BLM LIBRARY
88044015

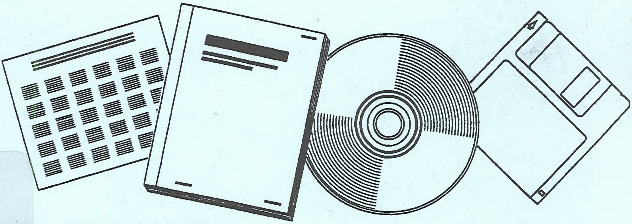
PB91153338

Information is our business.

NEUTRALIZATION OF ACID MINE DRAINAGE INFLUENCED LAKES BY ORGANIC ADDITIONS

SOUTHERN ILLINOIS UNIVERSITY AT EDWARDSVILLE
EDWARDSVILLE, IL

FEB 90



TD
899
.M5
B783
1990a

.S. DEPARTMENT OF COMMERCE
ational Technical Information Service

#35598298 ID 88044015 PB91-153338

TD
899
.M5
B783
1990a

A mining research contract report
FEBRUARY 1990

NEUTRALIZATION OF ACID MINE DRAINAGE INFLUENCED LAKES BY ORGANIC ADDITIONS

Contract J0178025
Richard B. Brugam
James Gastineau
Eric Ratcliff
Anna Stevens
Department of Biological Sciences
Southern Illinois University at Edwardsville
Edwardsville, Illinois 62026-1651

BUREAU OF MINES
UNITED STATES DEPARTMENT OF THE INTERIOR

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL
INFORMATION SERVICE
SPRINGFIELD, VA 22161

50272-101

REPORT DOCUMENTATION PAGE	1. REPORT NO.	2.	3. PB91-153338

4. Title and Subtitle
Neutralization of Acid Mine Drainage-Influenced Lakes by Organic Additions

5. Report Date
February 1990

6.

7. Author(s)
Richard B. Brugam, James Gastineau, Eric Ratcliff, Anna Stevens

8. Performing Organization Rept. No.

9. Performing Organization Name and Address
Southern Illinois University at Edwardsville
Biology Department
Edwardsville, IL 62026

10. Project/Task/Work Unit No.

11. Contract(C) or Grant(G) No.
(C) J0178025
(G)

12. Sponsoring Organization Name and Address
Bureau of Mines
Branch of Procurement - Washington
2401 E. Street N.W.
Washington, DC 20241

13. Type of Report & Period Covered
Final Report

14.
10/1/87 - 12/31/89

15. Supplementary Notes

16. Abstract (Limit: 200 words)

We conducted a series of 5 experiments in which 1 m diameter and 3 to 6 m long cylindrical enclosures were built in an acidic lake on a coal mine site. The enclosures extended from the lake surface to the sediment. We added limestone, phosphate, sewage sludge or wheat straw to the enclosures to test their neutralizing capabilities. Theory suggests that sewage sludge and wheat straw should be substrates for sulfate reduction by bacteria and that the production of H_2S and its precipitation as FeS should remove sulfuric acid from the water column. The limestone additions raised pH as expected. Straw additions supported sulfate reduction. Sulfate reduction was strongest where both lime and straw were added together. Straw additions produced the expected neutralization in our experiments, but neutralization was not permanent. We speculate that the productions of chelating substances from the decaying straw prevented the precipitation of FeS. Iron concentrations remained high in straw enclosures even though sulfide concentrations were also high.

17. Document Analysis a. Descriptors

Acid Mine Lakes, Sulfate Reduction, Acid Lake Neutralization

b. Identifiers/Open-Ended Terms

c. COSATI Field/Group

18. Availability Statement	19. Security Class (This Report)	21. No. of Pages
	20. Security Class (This Page)	22. Price

(See ANSI-Z39.18)

See Instructions on Reverse

OPTIONAL FORM 272 (4-77)
(Formerly NTIS-35)
Department of Commerce

FORWARD

This report was prepared by Southern Illinois University at Edwardsville under USBM Contract number J0178025. The contract was initiated under the Abandoned Minelands Reclamation Program. It was administered under the technical direction of the Environmental Division, Bruceton Research Center with Dr. Pamela McIntire acting as Technical Project Officer. Ms. Joyce Tillman was the contract administrator for the Bureau of Mines. This report is a summary of the work recently completed as a part of this contract during the period October 1, 1987 to December 31, 1989. This report was submitted by the authors on Feb. 28, 1990. It was reviewed and resubmitted on September 30, 1990.

We are especially indebted to the personnel at Peabody Coal Company's Fairview Heights, Illinois, Office and at Will Scarlett Mine. These include Mr. David MacDonald, formerly of the Fairview Heights office and Ms. Mary Siemtluz also at Fairview Heights. Mr. Phil Stafford, mine superintendent, and Mr. Robert Hill, environmental manager, at Will Scarlett Mine were especially helpful with on-site arrangements. We thank all for their help and cooperation. A first draft was reviewed by Patricia Ericksson and Pamela McIntire at the U.S. Bureau of Mines and by Alan Herlihy at the U.S. E.P.A.

We thank Michael Adams, Jairus Winfrey, Mark Gallyoun, Stuart Glover, and Kristen Ericksson -- students at SIU-Edwardsville -- for help in sampling and chemical analysis.

Reference to specific brands, equipment, or trade names in this report is made to facilitate understanding and does not imply endorsement by either Southern Illinois University at Edwardsville or the U.S. Bureau of Mines.

CONTENTS

Page

REPORT DOCUMENTATION PAGE.................................. 3
FORWARD.. 4
CONTENTS... 5
LIST OF ILLUSTRATIONS...................................... 6
EXECUTIVE SUMMARY.. 9
INTRODUCTION... 11
Surface Mine Lakes in the Midwest................ 11
Hypotheses about Lake Neutralization.............. 11
The Role of Sulfate and Iron-Reducing Bacteria.... 13
Lake Enclosure Experiments........................ 13
METHODS.. 16
Location.. 16
General Experimental Design....................... 16
Field Sampling.................................... 19
Laboratory Methods................................ 19
Quality Control................................... 20
Phytoplankton..................................... 21
Bacteria.. 21
Statistical Analysis.............................. 22
Experimental Conditions........................... 22
Quality and Sources of Additives.................. 23
RESULTS AND DISCUSSION 24
Experiment 2...................................... 24
Purpose 24
Additives................................... 24
Results..................................... 25
Discussion.................................. 26
Experiment 3...................................... 32
Purpose 32
Additives................................... 33
Results..................................... 33
Discussion.................................. 38
Experiment 4...................................... 38
Purpose 38
Additives................................... 38
Results..................................... 39
Discussion.................................. 51
Experiment 5...................................... 53
Purpose 53
Additives................................... 53
Results..................................... 54
Discussion.................................. 65
Experiment 6...................................... 65
Purpose 65
Additives................................... 70
Results..................................... 70
Discussion.................................. 80
GENERAL CONCLUSIONS.. 81
The Role of Iron in the Neutralization Process.... 81

The Fate of Reduced Iron and Sulfur in an Organically Loaded Lake.......................... 83
The Role of Organic Matter in the Natural Recovery of Mine Lakes.......................... 83
The Use of Artificial Organic Matter Additions to Encourage Neutralization........................ 84
RECOMMENDATIONS....................................... 85
REFERENCES... 87

LIST OF ILLUSTRATIONS

Figure Page

1. Research Location................................. 17
2. Experimental Apparatus............................ 18
3. Standing Stock of Net Acid Neutralizing Capacity in Experiment 2.............................. 27
4. Standing Stock of Net Acid Neutralizing Capacity in Experiment 2 (Continued).................. 27
5. Comparisons of total phosphorus concentrations among treatments in Experiment 2 on 5/3/88....... 29
6. Comparisons of acidity among treatments in Experiment 2 on 5/31/88.............................. 29
7. Comparisons of alkalinity among treatments in Experiment 2 on 5/31/88................... 30
8. Comparisons of pH between treatments in Experiment 2 on 5/31/88................................ 30
9. Variations in oxygen concentrations at 3 depths in the straw enclosure during Experiment 2.... 31
10. Standing Stock of Net Acid Neutralizing Capacity (ANC) in Experiment 3..................... 34
11. Standing Stock of Net Acid Neutralizing Capacity (ANC) in Experiment 3 (Continued).......... 34
12. Comparisons of sulfide concentrations among treatments in Experiment 3 on 7/6/88....... 37
13. Standing Stock of Net Acid Neutralizing Capacity (ANC) in Experiment 4..................... 40
14. Standing Stock of Net Acid Neutralization Capacity (ANC) in Experiment 4 (Continued).......... 40
15. Variations in oxygen concentrations at 3 depths in the straw enclosure during Experiment 4.... 43
16. Variations in sulfide concentrations at 3 depths in the straw enclosure during Experiment 4.... 43
17. Variations in sulfide concentrations at 3 depths in the lime/straw enclosure during Expt 4..... 44
18. Variations in pH at 3 depths in the straw enclosures during Experiment 4........................ 44
19. Variations in alkalinity at 3 depths in the straw enclosure during Experiment 4............. 45
20. Variations in acidity at 3 depths in the straw enclosure during Experiment 4............. 45
21. Variations in sulfate at 3 depths in the lime/straw enclosure during Experiment 4............. 46
22. Variations in sulfate at 3 depths in the straw

enclosure during Experiment 4............. 46
23. Variations in iron at 3 depths in the straw enclosure during Experiment 4............. 47
24. Variations in iron concentrations in the control enclosure during Experiment 4............. 47
25. Variations in iron concentrations at 3 depths in the straw enclosures during Experiment 4... 48
26. Comparisons of iron concentrations between treatments in Experiment 4 on 9/15/88...... 49
27. Comparisons of aluminum concentrations between treatments in Experiment 4 on 9/15/88...... 49
28. Comparisons among zinc concentrations in Experiment 4 on 9/15/88................... 50
29. Comparisons of manganese concentrations among treatments in Experiment 4 on 9/15/88...... 50
30. Standing Stock of Net Acid Neutralizing Capacity (ANC) in Experiment 5...................... 55
31. Standing Stock of Net Acid Neutralizing Capacity (ANC) in Experiment 5 (Continued).......... 55
32. Variations in sulfide concentrations at three depths in the straw enclosure during Experiment 5.............................. 58
33. Variations in sulfide concentrations at three depths in the lime/straw enclosure during Experiment 5........................ 58
34. Variations in sulfide concentrations in the control enclosure during Experiment 5...... 59
35. Variations in sulfide concentrations in the open lake during Experiment 5............. 59
36. Comparisons among treatments in Experiment 5 on 4/18/89.................................... 60
37. Comparisons of alkalinity among treatments in Experiment 5 on 4/18/89................... 60
38. Variations in acidity at 3 depths in the lime enclosure during Experiment 5............. 61
39. Variations in acidity at 3 depths in the lime/straw enclosure during Experiment 5... 61
40. Variations in acidity at 3 depths in the deep lime/straw enclosure during Experiment 5... 62
41. Variations in acidity at 3 depths in the straw enclosures during Experiment 5............ 62
42. Variations in acidity of the control enclosure during Experiment 5...................... 63
43. Variations in the acidity of the open lake during Experiment 5.............................. 63
44. Comparisons of sulfate concentrations among treatments in Experiment 5 on 4/18/89...... 64
45. Comparisons of aluminum concentrations among treatments in Experiment 5................ 64
46. Comparisons of iron concentrations among treatments in Experiment 5 on 5/9/89....... 66
47. Variations in iron concentrations at 2 depths in the lime enclosure during Experiment 5......... 67
48. Variations in iron concentrations at 2 depths

in the control enclosure during Experiment 5.......................... 67
49. Variations in iron concentrations at 2 depths in the lime/straw enclosure during Experiment 5.............................. 68
50. Variations in iron concentrations at 2 depths in the straw enclosure during Experiment 5.............................. 68
51. Variations in Secchi disc transparence in experimental enclosures during Expt. 5..... 69
52. Variations in Secchi disc transparency in experimental enclosures during Expt. 5..... 69
53. Standing Stock of Net Acid Neutralizing Capacity (ANC) in Experiment 6...................... 71
54. Standing Stock of Net Acid Neutralizing Capacity (ANC) in Experiment 6 (Continued).......... 71
55. Comparisons of oxygen concentrations among treatments in Experiment 6 on 8/1/89....... 74
56. Comparisons of acidity among treatments in Experiment 6 on 8/1/89..................... 74
57. Comparisons of sulfide concentrations among treatments in Experiment 6 on 8/1/89....... 75
58. Comparisons of pH among treatments in Experiment 6 on 8/1/89..... 75
59. Comparisons of alkalinity among treatments during Experiment 6 on 8/1/89..................... 76
60. Comparisons of aluminum concentrations among treatments in Experiment 6 on 8/1/89....... 77
61. Comparisons of zinc concentrations among treatments in Experiment 6 on 8/1/89.... .. 77
62. Variations in Secchi disc transparency in enclosures during Experiment 6............. 78
63. Variations in Secchi disc transparency in enclosures during Experiment 6............. 78
64. Comparisons between amounts of straw added to an enclosure and the maximum amount of Acid Neutralizing Capacity generated........... 79

EXECUTIVE SUMMARY

We used polyethylene enclosures to test the hypothesis that organic matter additions to acidic surface mine lakes would induce neutralization. We expected that organic additions would provide a substrate for bacterial sulfate reduction resulting in the removal of sulfuric acid from the water columns. We suspended six 1 meter wide cylindrical polyethylene enclosures from a floating dock in an acid lake. Each enclosure received a different additive or combination of additives. Six experiments were attempted. Experiment 1 was terminated early because of equipment failure.

In Experiment 2 we added calcium carbonate, phosphate, sewage sludge and straw to different enclosures. Two enclosures were reserved as controls for experimental manipulations. Calcium carbonate neutralized its enclosures as expected. We expected added phosphate to fertilize the lake producing decomposible organic matter. Fertilization failed because under acidic conditions phosphate precipitates with iron and aluminum preventing any fertilization effect. We expected sewage sludge to provide an organic substrate for sulfate reduction, but the sewage sludge proved to be too stable to provide a substrate for decomposition. By the end of the experiment, straw provided a slight neutralization effect.

Experiment 3 was performed to test if inclusion of anaerobic deep water within the enclosures would enhance neutralization. Because of low levels of organic additions, neutralization did not occur.

In Experiment 4 organic matter (straw) additions at levels higher than those of previous experiments continued until clear neturalization effects were seen. We added limestone with the organic matter to test if sulfide reduction is pH limited. We found strong sulfide reduction in all enclosures with added straw. The straw enclosures had increased pH and alkalinity but acidity reduction was small. Furthermore, iron concentrations did not decline in the enclosures with significant sulfate reduction. As a result, although sulfate reduction occurred, it produced only temporary neutralization. As soon as the water column became aerobic, pH dropped.

In Experiment 5 we tried to enhance sulfate reduction by trapping straw near the lake bottom. We speculated that the lake sediment might provide a location for long-term sulfate reduction. Results were similar to experiment 4 because, although sulfide reduction produced acid neutralizing capacity, the straw-dosed enclosures soon re-acidified.

In Experiment 6 we tested dosage rates by adding different

amounts of straw to enclosures. We found that the amount and duration of neutralization varied with the dosage.

We conclude from our experiments that sulfate reduction is a process that can be used to neutralize acid mine lakes, but that the process, as we employed it, only resulted in temporary neutralization. Other investigators (30,31,15,16) have shown that sulfate reduction only becomes permanent if the resulting sulfide is removed from the water column as H_2S or FeS. We speculated that in our experiments the water columns were too long to allow significant outgassing of sulfur as H_2S and that the presence of organic chelators prevented precipitation of FeS. Our results emphasize that for permanent neutralization of acid waters, sulfate reduction is not enough. The resulting sulfide must be removed from the water column and permanently sequestered.

INTRODUCTION

Surface Mine Lakes in the Midwest

Lakes are a more or less desirable byproduct of surface mining for coal in the American Midwest. Alkaline surface mine lakes can be a significant environmental amenity and can be used for fishing and water supply (24). Acid lakes do not support fish, are useless for water supply without extensive treatment and produce pollution effluents at their outfalls. Both kinds of lakes are formed when abandoned mine pits fill with groundwater. The resulting lakes are deep, steep-sided and contain water with extremely high levels of dissolved solids (35,10). Many mine lakes are extremely acid with pH's as low as 2.0. Low pH's are caused by the weathering of sulfide minerals (pyrite and marcasite) exposed in the minespoil surrounding the lake. The weathering of pyrite follows equation a (from 22) and results in the production of large amounts of sulfuric acid.

a) $FeS_2 + 3.75\ O_2 + 3.5\ H_2O \text{--->} Fe(OH)_3 + 2SO_4^{-2} + 4H^+$

In addition to its high sulfuric acid content and low pH, acid mine drainage also contains high concentrations of many heavy metals (17). In Illinois, 8% of the 14,000 acres of surface mine impoundments are "adversely affected or undesirable" because of acid inputs (23,14). In other midwestern areas like southern Indiana (7,35), mine overburdens are not as rich in acid neutralizing materials as Illinois, so acid lakes are even more common.

Although the current Federal Surface Mining and Reclamation Control Act (P.L. 95-87) forbids the creation of all "final cut" impoundments (whether acid or alkaline) and mandates regrading to fill large water bodies, many lakes remain on sites mined before the passage of the mine law in 1977. The poorest water quality is found in lakes on "abandoned mine lands" where mining operations ceased before August 3, 1977.

Hypotheses About Lake Neutralization

A number of investigators (6, 8, 5) have shown that acidic mine lakes neutralize over time. The cause of the neutralization is unclear but it is important to discover the mechanism of acid lake neutralization so that ways can be found to accelerate the process.

Some investigators have suggested that within-lake sulfate reduction plays a major role in the neutralization process. King *et al.* (22) argued that neutralization occurs as the lake watershed becomes revegetated and contributes organic matter in the form of leaves and twigs to the lake. This organic matter becomes a substrate for sulfate-reducing bacteria which produce H_2S in the lake water column. King *et al.* (22) tested their hypothesis using laboratory microcosms and demonstrated that organic matter additions to acid mine drainage do support sulfate reduction and neutralization. According to King et al. (22) the H_2S produced would be outgassed from the lake surface removing H^+ from the lake. Direct outgassing of H_2S from the surface of mine lakes seems unlikely to occur at any significant rate because in most lakes the H_2S is prevented from contact with the air by 2 to 10 m of oxygenated water. Any H_2S mixed into this zone would be oxidized back to H_2SO_4.

A more appropriate hypothesis concerning internal neutralization of sulfuric acid in lakes is presented by Schindler et al. (30). Their evidence shows that reduction of sulfate to sulfide and storage of this sulfide in the sediment as FeS is a source of neutralization. The sediment and hypolimnion are the probable locations of this reaction in surface mine lakes. More recently Schindler et al. (31) found that sediment sulfate reduction contributed 39 $meq/m^2/yr$ to the acid neutralizing capacity (alkalinity) of an experimentally acidified lake in Ontario.

Anderson and Schiff (2) showed that in acid rain influenced-lakes permanent neutralization was produced by iron and sulfate reduction and precipitation as FeS. The reduction of sulfate to H_2S would only produce temporary neutralization. The reduction of iron consumes H+ according to equation b.

b) $CH_2O + 4FeOOH + 8H^+ \rightarrow CO_2 + 4Fe^{++} + 7H_2O$

Although the reduction of SO_4^{--} consumes H^+ (equation c), the H+ is released again as the H_2S is reoxidized or the S-- is precipitated as FeS (equation d).

c) $2CH_2O + SO_4^{--} + 2H^+ \rightarrow 2CO_2 + H_2S + 2H_2O$

d) $Fe^{++} + H_2S \rightarrow FeS + 2H^+$

The reduction of iron is thus a requirement for neutralization of acid lakes by FeS precipitation. If iron reduction and precipitation do not occur, high pH will be maintained only as long as H_2S exists. As soon as the water column becomes aerobic again, the H_2S will be oxidized to H_2SO_4. The neutralization process as seen by Anderson and Schiff (2) converts FeOOH into FeS while releasing the OH^- into the water column.

In acid mine drainage, sulfate reduction might not result in neutralization by exactly the mechanism proposed by Anderson and Schiff (2) because a significant fraction of the iron in acid mine drainage is present as Fe^{++} already (26). In this case, sulfate reduction and precipitation should remove the Fe^{++} that produces a large part of the mineral acidity in acid mine drainage.

The Role of Sulfate and Iron-Reducing Bacteria

Because of their importance in the neutralization process, it is important to understand the physiology of the sulfate reducing bacteria that inhabit lakes. These organisms are obligately anaerobic heterotrophs of a number of genera which reduce large quantities of sulfate to sulfide in a process called dissimilatory sulfate reduction. The bacteria use organic carbon as an electron donor and sulfate as an electron acceptor in a manner similar to aerobic heterotrophs using organic carbon and oxygen. For the sulfate reducers, oxygen is replaced by sulfate in respiration. Because oxygen-based respiration is more energy efficient than sulfate-based respiration, sulfate-reducing bacteria are only active where there is not enough oxygen to support aerobic heterotrophs. (27)

Other bacteria and algae also reduce sulfate to sulfide under aerobic conditions, but they engage in the process only to supply reduced sulfur for essential biochemical compounds. This process is called assimilatory sulfate reduction and produces only small quantities of reduced sulfur. (39)

Iron-reducing bacteria behave similarly to sulfate-reducing bacteria. Instead of using sulfate as an electron acceptor, they use Fe^{+++}. Like sulfate-reducers, they are obligate anaerobes. (37).

Baas-Becking *et al*. (4) found that dissimilatory sulfate reduction is limited to environments with pH>4.2 environments. Their results are based on a survey of the literature for data in which the redox potential and pH were reported for environments in which sulfate reduction was occurring. Schuurkes and Kok (32) tested rates of sulfate reduction in laboratory studies of lake sediment incubated at different pH's and found that the reaction was slowed at pH<5.0 for samples from acid lakes and pH<6.0 for samples from circumneutral lakes.

Lake Enclosure Experiments

We tested the validity of the theory that sulfate reduction would promote acid mine lake neutralization by adding organic matter to an acidic mine lake. Theory would suggest that additional organic matter should accelerate the neutralization of the lakes by stimulating bacterial reduction reactions. Unfortunately, the direct addition of organic matter to a whole lake presents a number of difficulties. The most important difficulty is that of experimental controls. To truly test the effects of organic matter addition, one would need two exactly paired lakes -- one for the experimental modification and the other for a control. An exact pairing of lakes is virtually impossible.

The use of a whole lake for such an experiment also presents the problem that multiple management strategies cannot be tested side by side. For example, one would have difficulties testing different sources of organic matter as potential neutralizing agents. To conduct a test of the organic neutralization hypothesis on whole lakes would also be logistically difficult because it would require the use of a large number of lakes and consume large amounts of material.

These problems with whole-lake manipulation experiments can be mitigated by using enclosures of lake water. Enclosures are portions of the lake that are walled off from the main body of water and which can be manipulated separately to test hypotheses about lake processes. In most cases these enclosures have been either plastic bags containing lake water (28) or tubes constructed of plastic film that stretch from the lake surface to the sediment (25).

Havens and DeCosta (13) and Wilcox and DeCosta (40) tested the effects of acidification and nutrient additions to lakes in enclosures suspended in a West Virginia lake. Their enclosures were 1750 liter plastic bags which were closed at the bottom. The use of enclosures allowed the investigators to vary experimental protocols between small replicate bodies of lake water. One bag was reserved as a control and the others were either acidified or fertilized or both. They found that primary productivity was unaffected by acidification except for a brief period of "pH shock" at the beginning of the experiment when growth rates were reduced.

Lund (25) used much larger (45.5 m diameter) enclosures of butyl rubber that reached from the surface to the bottom of Blelham Tarn in England. He added phosphate and silicate to the enclosures and examined the effect of addition of these nutrients on phytoplankton growth and succession.

Shapiro (34) used polyethylene enclosures in Minnesota lakes to show the effect of additions of CO_2, phosphorus, and nitrogen on nuisance blooms of blue-green algae.

Schindler *et al.* (31) used enclosures in addition to whole lake manipulations to test the effect of acidification with sulfuric acid on lakes in western Ontario. Their purpose was to examine the effects of acid precipitation on lakes. They found that the added sulfate was quickly reduced to sulfide by sulfate-reducing bacteria. The sulfide was eventually precipitated as ferrous sulfide. This process raised the pH of the enclosed water column.

Schiff and Anderson (29) continued Schindler *et al.*'s (2) work using enclosures that were open to the sediment. They added sulfuric acid to the enclosures to maintain a pH of 5.0 to 5.2 -- pH's normally encountered in an acid precipitation-influenced lake. They found that additions of acid had to continue in order to maintain the low pH. Schiff and Anderson (29) hypothesized that the sediment continuously consumed acid.

In contrast to Schiff and Anderson (29), who added acid to a neutral lake containing natural organic matter, we conducted experiments in which we added organic matter to a lake that is already acid. We tested the effectiveness of organic matter in raising pH and reducing acidity in Pit #8 of Will Scarlett Mine, an acidic surface-mine lakes. All tests were performed in large polyethylene enclosures similar to those of Schiff and Anderson (29).

METHODS

Location

Pit #8 of the Will Scarlett Coal Mine, Carriers Mills, Illinois, was chosen for our experiments because it is an acidic mine lake with an appropriate range of depths (see Figure 1). Peabody Coal Company had already done a thorough morphometric and limnological survey of the lake (20). The lake is a final cut impoundment with three inclines whose depth grades from 0 to 22.9 m. It was formed when mining ceased in 1972. The pH of the main pit was 4.2 on March 21, 1988, but had increased to 6.1 by September, 1989.

We decided to locate the experimental enclosures in the middle incline of the lake. It is separated from the main lake by a shallow sill (Fig 1). The water at the head of the middle incline is more acid (pH<3.1) than the main lake (pH=6.1). Apparently, the middle incline has a separate source of acid mine drainage which is prevented from mixing with the main lake by the sill.

General Experimental Design

Experiments were performed in cylindrical polyethylene enclosures 3 to 6 m long by 1 m wide held open with large plastic hoops. Six cylinders (Fig. 2) were suspended from a floating dock in the middle incline of Pit #8 (Fig. 1). The tops of the cylinders were open to the air and the bottoms were open and sunk in the mud of the lake bottom. This configuration allowed natural exposure of the water column within the enclosures to the sediment and to the atmosphere.

The advantage in using enclosures for limnological experiments is that small regions of a lake can be partitioned off from the main lake for experimentation. These "enclosures" allow the establishment of controls to the experimental manipulations. They also reduce the expense of experiments by reducing the amount of additives needed. In the case of experiments where the possibility of temporary environmental degradation exists, a smaller portion of the environment is placed at risk.

A disadvantage of enclosures is that, although the polyethylene cylinders reduce interchange with the open lake, they do not completely prevent it. One would expect that effects of treatments would be of shorter duration in enclosures than they might be in a whole lake because of this interchange.

Five complete experiments numbered 2 to 6 were performed during the spring and summer of 1988 and 1989. Experiment 1 was

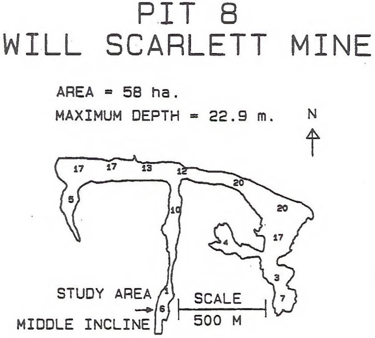


Figure 1: Research Location

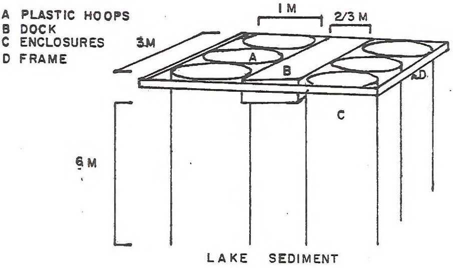


Figure 2: Experimental Apparatus

begun in April, 1988, but was terminated early when the enclosures floated out of the lake sediment because of rising lake water levels. Experiment 1 will not be discussed further.

During each experiment, different materials were added to the lake water confined in the enclosures as tests of their ability to stimulate sulfate-reduction. In all cases, chemical sampling of enclosures began within one hour of the first application of additives.

Field Sampling

Water samples were taken from the top, middle and bottom of each enclosure and from the open lake. The precise depth of the samples varied between experiments because enclosure lengths varied from 3 to 6 m. Three kinds of water samples were taken at each depth using a Kemmerer sampler. One sample was placed in a 1 liter, acid washed, polyethylene bottle to be used for general chemical analyses. Temperature measurements of the sample were made in the polyethylene bottle immediately after collection with a field thermometer. Within 1 hour of sampling, pH was measured in the polyethylene bottles using a Corning glass-electrode field pH meter. Another sample was placed in a clear 300 ml B.O.D. bottle with 1 ml of 1N zinc acetate solution for sulfide analysis. A third sample was collected in a darkened 300 ml B.O.D. bottle for oxygen analysis by the Winkler method. This sample was treated with manganous sulfate and alkaline azide reagent to fix oxygen concentrations in the field (1). The Winkler oxygen method was chosen over the easier oxygen electrode method because the acid water of Pit #8 corroded electrodes making them unreliable.

Secchi disc measurements were made to determine water clarity on several occasions during experiments 5 and 6. These measurements were made down each enclosure with a standard limnological Secchi disc.

Laboratory Methods

In the laboratory, samples were analysed using the techniques of Standard Methods (1). Analysis began immediately after our return from the field.

Samples were first analysed for alkalinity using potentiometric titration to a pH 4.4 endpoint with 0.02 N H_2SO_4. Alkalinity was measured in all samples having a lab pH>4.4. Analyses were done within 6 hours of sample collection.

Acidity of low-pH samples was measured after boiling with H_2O_2. Samples were titrated potentiometrically with 0.02 N NaOH to an endpoint of pH 8.3. Analyses were done within 6 hours of sample collection.

H_2SO_4 was added to the field-fixed oxygen samples in the laboratory. Oxygen was measured by iodimetric titration with thiosulfate.

Likewise, total sulfide was measured by iodimetric titration with thiosulfate using samples from the zinc acetate-dosed B.O.D. bottles. Sulfide analysis was done within 24 hours of sample collection.

Within a week of sample collection, sulfate was determined turbidimetrically using barium chloride to precipitate sulfate. Analyses were performed on small aliquots of lake water (1 to 3 ml) because of high sulfate concentrations.

Total phosphorus was measured on unfiltered samples that had been digested with persulfate in an autoclave. Measurement of total phosphorus was performed spectrophotometrically with the phosphomolybdate-blue method. Ascorbic acid was used as the reducing agent. Total phosphorus determinations were only made during 1988.

One hundred milliliter aliquots were removed without filtering from the raw water samples for analysis of cations. One milliliter of concentrated HNO_3 was added to the 100 ml aliquots to keep cations in solution. KCl was added to the 1988 samples to bring the final concentration of K^+ to 2 mg/l. This addition was made to prevent ionization problems when Al was measured by atomic absorption. The aliquots were collected and stored at 4° C until analyses could be performed (about 9 months later).

Cation analyses were performed using atomic absorption spectrophotometry. Cations analysed were Fe, Mn, Al, and Zn. Analyses of Cu, Cd and Pb were attempted, but concentrations were below detectable levels for the techniques used. Except for Al, all metals were analysed using an air-acetylene flame. For Al analysis a nitrous oxide-acetylene flame was used. A Varian 1000 atomic absorption spectrophotometer was used for all metal analyses during 1988. A Varian model AA10 was used during 1989.

During the second year of the experiment aluminum measurements were done using the Eriochrome Cyanine RC spectrophotometric method from Standard Methods (1). This method was chosen because it is sensitive within the range of values found in our samples (approximately 2 mg/L). The optimum range of the atomic absorption method is somewhat above the mean of our samples.

Quality Control

In order to ensure reproducibility in chemical analyses, an artificial acid mine drainage solution was produced in the laboratory. The solution consisted of WC algal growth medium (11) without trace metal additions. To this solution we added 2 mg/l of each of the metals in the form of atomic absorption standard solutions prepared by Fisher Scientific Company. We added the metals which we routinely measured in Pit #8 (Table 1).

Table 1: Composition of artificial acid mine drainage. Errors are one standard deviation.

Constituent	Amount Added	Amount Measured	Repetitions
H^+oS H_2SO_4	36 meq/l	40 ±3 meq/l	20 Not in range
Al	2 mg/l	2.05±.53 mg/l	20
Fe	2 mg/l	2.59±.40 mg/l	18 Not in range
Zn	2 mg/l	1.68±.13 mg/l	17 Not in range
Mn	2 mg/l	1.87±.13 mg/l	17
Cd	2 mg/l	1.92±.04 mg/l	9 Not in range
SO_4^{--} (includes $MgSO_4$ + H_2SO_4)	1728 mg/l	2100 ±499 mg/l	22
H_3BO_3	6 mg/l	not measured	
$MgSO_4.7H_2O$	37 mg/l	not measured	
K_2HPO_4	9 mg/l	not measured	
$CaCl_2$	40 mg/l	not measured	

Four liters of artificial acid mine drainage were prepared at the beginning of the 1989 field season. Analyses of the artificial acid mine drainage were made on aliquots from the original 4 liters at the same time as the samples from Pit #8. Analyses were repeated each week that we sampled the lake.

Measurements of 4 chemical variables were not within 1 standard deviation of the expected value (not in range on Table 1). Differences can probably be attributed to inaccuracies in the original preparation of the artificial acid mine drainage.

Phytoplankton

Phytoplankton samples were taken from the middle depth of each enclosure and from the open lake for experiments 2 through 4. Samples were fixed with Lugol's (I_2-KI) solution and counted using the inverted microscope technique of Utermöhl (33).

Bacteria

Samples of water and sediment from Experiment 4 were incubated anaerobically in API (American Petroleum Institute) Broth. API Broth contains only sulfate, lactose, ferrous ion, and an inorganic nitrogen source. It is an indicator medium for sulfate-reducing bacteria (3, 9).

Anaerobic conditions were maintained in the cultures by using a "Gas Pak". This is an air-tight vessel into which the inoculated cultures were placed. The vessel contains an H_2 gas generator and a palladium catalyst which removes oxygen from the air as water. Incubation of the cultures was at 20° C.

Statistical Analysis

A major statistical question of our work was "Are the differences between treatments real?". To test this question we used the Wilcoxon Signed Rank Test (38). This test uses paired samples in which one set of samples is subtracted from the other. For our experiments we paired samples at each depth and each date between treatments. For example, in comparisons between the control and straw enclosures samples at a given depth and sample date were paired. The sample from the straw enclosure for a particular water quality parameter were subtracted from the control sample with which it was paired. The absolute values of the differences were ranked and the rank of positive differences were summed. Likewise, the ranks of the negative differences were also summed. A test statistic (Z) was calculated and all experimental pairs were tested.

The advantage of the Wilcoxon Signed Rank Test is its lack of assumptions about statistical distributions of the samples. An alternative test would be the paired sample ANOVA, but this assumes that the data are normally distributed and have equal variances -- assumptions that are too restrictive for our data.

Experimental Conditions

Because of variations in lake conditions, the circumstances of each experiment were slightly different. Until August 24, 1988, the water of the middle incline was strongly stratified. The surface water, though acid (acidity = 70 mg/l $CaCO_3$, pH= 3.0) did not have as high a concentration of dissolved solids as the deep water. The water at depths greater than 5 m was, however, much more strongly influenced by acid mine drainage (acidity = 500 mg/l $CaCO_3$, pH=2.7). Experiment 2 was performed in shallow water (less than 4m) so there was no effect of the acid mine drainage layer on the results. Experiment 3 was performed in 6 m of water during the time of stratification so the acid mine drainage layer may have been important in the results. Experiment 4 was also performed in 6 m of water, but by the start of the experiment, the layer had mixed into the rest of the lake.

In the second year, experiments were again performed in shallow water (3m) because we feared that long polyethylene cylinders would allow more interchange with the surrounding lake water than short ones. During the second year, the middle incline was also stratified below 5 meters. In contrast with 1988, the lake remained stratified throughout the experiments, but the enclosures were located at 3 m -- shallower than the stratified layer. The deepest layers of the enclosures had higher dissolved solids than the shallow layers but the difference was not as strong as it had been in 1988.

Quality and Sources of Additives

An attempt was made during these experiments to use materials that would be normally available to a coal mine operator. On a few occasions chemically pure laboratory reagents were used. Additives and sources are listed below.

$K H_2PO_4$ - Fisher Analysed reagent grade chemical

Chalk - $CaCO_3$ - Fisher Analysed reagent grade chemical - labeled Precipitated chalk.

Super phosphate fertilizer

Straw - Wheat straw

Hydrated Lime - $Ca(OH)_2$ packaged in bulk for agricultural purposes

Limestone - $CaCO_3$, $MgCO_3$ and silicate rock purchased loose from local agricultural supply store

Sewage Sludge - Anaerobic digester sludge taken from SIU-Edwardsville sewage treatment plant's drying beds

Mesh bags - .5 cm width mesh

RESULTS AND DISCUSSION

In this section each experiment will be discussed in turn. We will present the purpose of the experiment, the additives used, the results we obtained, and a brief discussion of the significance of each experiment.

Experiment 2   4/27/88 to 6/22/88

Purpose

Experiment 2 was our first successful experiment. We planned the additives to test our initial hypotheses about mine lake neutralization.

Wheat straw was used as an additive because it was expected to mimic the natural organic matter that would accumulate in a lake as it matures. King et al. (22) have suggested that this process is important in the natural recovery of surface mine lakes.

Anaerobic digester sludge was used as another organic additive because it is an organic material that might be used by mine operators to enhance the natural neutralization process.

Phosphate was added in a similar effort to enhance the natural neutralization process. The within-lake production of organic matter by plant growth is limited by the availability of phosphorus in lakes uninfluenced by acid (19). We supposed that fertilization of the lake with phosphate would result in the production of organic matter that would serve as a substrate for sulfate reducing bacteria.

Finally, $CaCO_3$ was added to one enclosure to examine the effects of the neutralization technique that is normally used by mine operators. Liming was compared with organic matter additions as a neutralization technique.

Two enclosures and the open lake itself served as controls for our experiment. The two control enclosures received no additives. The open lake samples were used to test if the enclosure process itself changed the responses of the lake.

Additives - Experiment 2 (Table 2)

Both sewage and straw additions were chosen to neutralize their respective enclosures simply by the HCO_3^- that would be generated when the organic matter they contained was degraded by bacteria. For our calculations, we assumed that the organic matter was mostly glucose. This is a reasonable assumption for straw with its high cellulose content, but it is likely to be an oversimplification for sewage sludge with its complex mixture of organic matter. The sewage additive was assumed to be 50%

organic matter.

Phosphate (as KH_2PO_4), in contrast, was added in gross excess of the agents expected to produce eutrophication. The expected final concentration of PO_4 in the phosphate enclosure was 10 mg/l. This high concentration was chosen to produce high rates of photosynthesis and organic matter production.

$CaCO_3$ in the form of chalk was added to the lime enclosure in an amount just large enough to remove acidity in the lime enclosure. No excess was added.

The open lake was sampled because of the concern that the presence of the cylinders might modify the conditions of the experiment. Samples were taken at the same depths as the samples from within the cylinders.

Near the end of experiment 2, because pH had not risen, it became apparent that too little organic matter had been added to the enclosures. On 5/31/88 additional straw, sewage sludge, and chalk were added to the appropriate enclosures (Table 2).

Table 2: Additives for Experiment 2 - April 27 to June 22, 1988 - 8 weeks - 4 m Depth

	Date of Addition	
Treatment	3/22	5/31
Control (C1)	Nothing	Nothing
Control (C2)	Nothing	Nothing
Lime	Chalk .46 kg	Chalk .90 kg
Phosphate	KH_2PO_4 .55 kg	Nothing
Sewage (Sew)	Sludge 1.6 kg	Sludge 7.5 kg
Straw	Straw .82 kg	Straw 3.0 kg
Open	Open lake measurements	

Results - Experiment 2

Before discussing neutralization processes, it is first necessary to establish criteria with which to judge the effectiveness of additives in reducing acidity in surface mine lakes. One criterion is the change in acid neutralizing capacity (ANC) caused by particular additives. ANC is measured by alkalinity and acidity titrations. Alkalinity directly measures ANC whereas acidity can be seen as negative ANC because for acidity to be removed, an equal amount of ANC must be supplied. Net ANC can thus be calculated as alkalinity minus acidity. We can say that some neutralization has occurred if there is an increase in net ANC even though net ANC is still negative (i.e. alkalinity is less than acidity).

A problem with using net ANC for comparison between experimental enclosures is that each enclosure is not homogeneous. There is significant variation of concentrations of ions with depth. To solve this problem we can calculate a "standing stock" of net ANC which is simply the total amount of net ANC in the enclosures. This number is calculated by multiplying the volume of water at each depth range times the net ANC in meq/l and summing over all depths. The result is a single number which can be used to compare treatments on each sampling day.

When comparisons of standing stock of net ANC are made for experiment 2 it is apparent that, as expected, additions of $CaCO_3$ increased the standing stock of net ANC (Figs 3 and 4). Increases also occurred after more $CaCO_3$ was added to the lime enclosure on 5/31/88 (week 5). The additions of KH_2PO_4 removed ANC (i.e. increased acidity). The amount of increase in acidity only slightly exceeded the amount expected from the H^+ added with the KH_2PO_4. Other treatments did not change the standing stock of net ANC. The open lake Net ANC was the same as the enclosures - except for an anomalous decline which occurred in the 4th week.

Another way to measure the effectiveness of additives is to test the statistical significance of differences between treatments. In this case, measurements at different depths in enclosures need not be summed as they were to measure standing stock of net ANC. The Wilcoxon Signed Rank Test is a non-parametric analogue to an ANOVA. It was applied to the data from experiment 2 (Table 3).

As expected, lime and phosphate enclosures show the most difference from the controls. Bar graphs are taken from May 3, 1988, a representative week. The phosphate enclosure was higher in total phosphorus and acidity than the controls (Fig. 5 and 6). The lime enclosure was higher in alkalinity and pH than the controls (Figs. 7 and 8). The pH of the straw enclosure was slightly higher than controls (Fig. 8) while its oxygen levels were lower (Fig. 9). When the data are examined more closely, it is apparent that the last weeks of the experiment have the lowest oxygen concentrations (Fig. 9). These strongly influence the results of the Wilcoxon Signed Rank Test. The low oxygen concentrations occur only after the addition of 3.0 kg of new straw to the enclosure on 5/31/88.

Discussion - Experiment 2

In experiment 2 the organic matter additions produced no neutralization until we added additional material on May 31. It was apparent that the amount of straw added at the beginning of the experiment was too small to produce sulfate reduction, but that the new straw added on May 31 began the process of neutralization.

Sewage sludge had no effect on neutralization even when extra material was added on May 31. Apparently, the sludge we used was

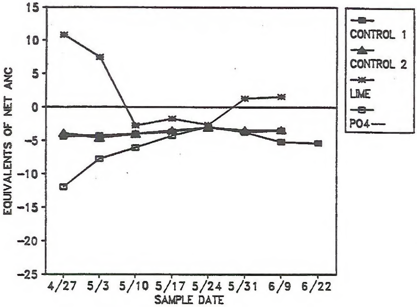


Figure 3: Standing Stock of Net Acid Neutralizing Capacity in Experiment 2.

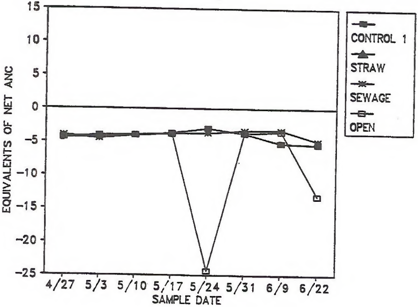


Figure 4: Standing Stock of Net Acid Neutralizing Capacity in Experiment 2.

Table 3: Wilcoxon Signed Rank Test on Experiment #2. Z statistics are listed.
* .05<P<.1 **.01<P<.05 ***P<.01

Temperature

	C1	C2	Lime	PO4	Straw	Sewage	Open
C1		-1.27	-0.55	-1.33	-2.04 **	-0.94	-0.32
C2			-1.78 *	0	-1.1	-0.81	-0.11
Lime				-1.68 *	-2.4 **	-1.58	-0.73
PO4					-0.84	-0.81	-0.07
Straw						-1.78 *	-0.85
Sewage							-0.68
Open							

pH

	C1	C2	Lime	PO4	Straw	Sewage	Open
C1		-0.25	-3.82 ***	-0.24	-2.69 ***	-1.76 *	0.59
C2			-4.02 ***	-0.53	-2.17 **	-0.75	-0.39
Lime				-4.02 ***	-3.82 ***	-3.92 ***	-4.01 ***
PO4					-2.53 **	-1.41	-1.08
Straw						-2.68 ***	-2.63 ***
Sewage							-1.22
Open							

Sulfide

	C1	C2	Lime	PO4	Straw	Sewage	Open
C1		0	0	0	-1.34	0	0
C2			0	0	0	0	0
Lime				0	0	0	0
PO4					0	-1.34	-1.34
Straw						0	0
Sewage							0
Open							

Alkalinity

	C1	C2	Lime	PO4	Straw	Sewage	Open
C1		0	-3.06 ***	0	0	0	0
C2			0	-3.06 ***	0	0	0
Lime				-3.06 ***	-3.06 ***	-3.06 ***	-3.06 ***
PO4					0	0	0
Straw						0	0
Sewage							0
Open							

Oxygen

	C1	C2	Lime	PO4	Straw	Sewage	Open
C1		-0.16	-2.58 ***	-0.83	-3.6 ***	-1.88 *	-1.84 *
C2			-2.35 **	-1.03	-2.8 ***	-2.42 **	-2.21 **
Lime				-2.73 ***	-2.35 **	-3.53 ***	-0.2
PO4					-2.84 ***	-0.56	-2.13 **
Straw						-3.95 ***	-1.59
Sewage							-3.46 ***
Open							

Sulfate

	C1	C2	Lime	PO4	Straw	Sewage	Open
C1		-1.33	-0.36	-0.7	-0.36	-0.93	-0.06
C2			-1.42	-1.93 *	-0.89	-2.03 **	-1.23
Lime				-0.15	-0.15	-0.86	-0.35
PO4					-0.14	-0.31	-0.83
Straw						-0.41	-0.19
Sewage							-1.35
Open							

TOTAL P

	C1	C2	Lime	PO4	Straw	Sewage	Open
C1		-1.14	-2.39 **	-3.03 ***	-0.62	-1.42	-1.45
C2			-1.05	-2.94 ***	-0.45	-1.92 *	-0.1
Lime				-2.9 ***	-1.5	-2.34 **	-1.24
PO4					-3.29 ***	-2.42 **	-3.1 ***
Straw						-1.59	-0.78
Sewage							-2.13 **
Open							

Acidity

	C1	C2	Lime	PO4	Straw	Sewage	Open
C1		-1.61	-3.98 ***	-2.51 **	-1.99 **	-1.4	-0.47
C2			-4.02 ***	-3.4 ***	-0.65	-0.65	-0.64
Lime				-3.98 ***	-3.92 ***	-4.02 ***	-4.02 ***
PO4					-3.15 ***	-3.18 ***	-1.96 **
Straw						-1.81 *	-1.48
Sewage							-0.83
Open							

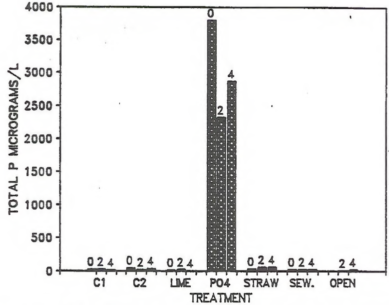


Figure 5: Comparisons of total phosphorus concentrations among treatments in Experiment 2 on 5/3/88. Numbers on columns are depths in enclosures.

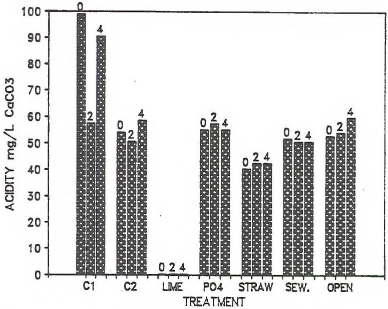


Figure 6: Comparisons of acidity among treatments in Experiment 2 on 5/31/88. Numbers on columns are depths in enclosures.

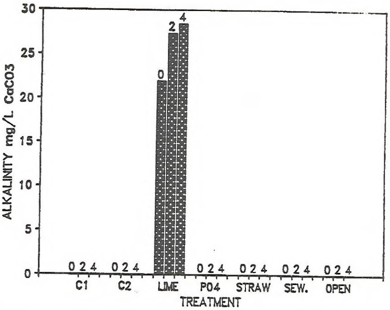


Figure 7: Comparisons of alkalinity among treatments in Experiment 2 on 5/31/88. Numbers on columns are depths in enclosures.

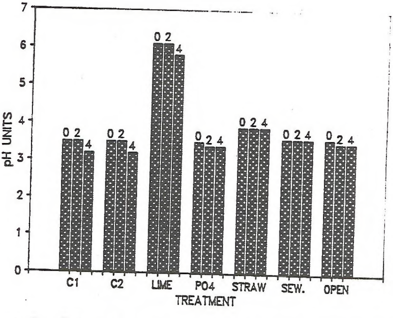


Figure 8: Comparisons of pH between treatments in Experiment 2 on 5/31/88. Numbers on columns are depths in enclosures.

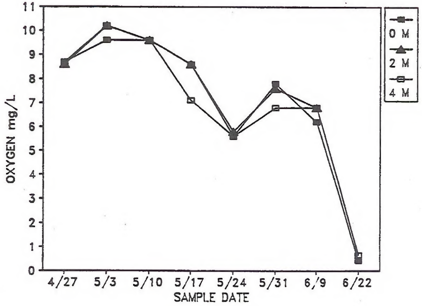


Figure 9: Variations in oxygen concentrations at 3 depths in the straw enclosure during Experiment 2.

too stable to provide enough easily decomposible organic matter to encourage neutralization.

The phosphate additions had just the opposite effect from that hypothesized. The added phosphate immediately precipitated producing a milky turbidity in the enclosure. It is likely that the precipitate was aluminum phosphate. This compound precipitates readily at low pH (39, 37). The additions of phosphate as KH_2PO_4 resulted in an increase in water column acidity as measured by the standing stock of net ANC.

$CaCO_3$ additions neutralized the enclosure as expected, but neutralization was not permanent. In a few weeks the pH and acidity of the lime enclosure approached that of the surrounding lake water. This result suggests that the enclosures may not be entirely leak-proof. It is likely that there is some interchange of water through the enclosure. The bottom probably allows some seepage of lake water into the enclosure.

The control enclosures seem to be adequate to control for effects resulting from the construction of the polyethylene cylinders. These effects seem to be small because the Wilcoxon Signed Rank Test only detected significant differences between the control enclosures and the open lake for oxygen concentrations.

Although experiment 2 showed only minor amounts of sulfide production, it did demonstrate that phosphate additions do not increase lake trophic status in acidic surface mine lakes. The high levels of dissolved aluminum and iron in these lakes could effectively compete with phytoplankton for phosphate. The aluminum and iron sequester added phosphate before phytoplankton can absorb it.

In this experiment, however, phosphate was added in huge excess of the amounts that could possibly be precipitated by aluminum or iron (assuming the formation of $AlPO_4$ or $FePO_4$). The standing stock of iron in the enclosures was 151 mmoles/L. The standing stock of aluminum was 346 mmoles/L. We added 4044 mmoles of PO4 . There should have been huge amounts of phosphate remaining to produce an algal bloom. It is likely that some other factor like low pH or nutrient limitation prevented the production of an algal bloom.

Experiment 3 - 6/22/88 to 8/17/88

Purpose

Because the small organic addition originally planned for experiment 2 failed to produce the strong neutralization effect we hoped for, we increased organic loading in experiment 3 by adding amounts of straw approximately equal to the amounts added at the end of experiment 2 (4/27/88). We also expected that the inclusion of the deep, anaerobic acid mine drainage from lake

depths >4m would enhance neutralization. For this reason we moved the experimental apparatus to a 6m deep location.

Additives - Experiment 3 (Table 4)

As with experiment 2, two control enclosures were designated. The lime enclosure received hydrated lime ($Ca(OH)_2$) in an amount expected to assure neutralization of the acidity trapped in the enclosure. We changed to hydrated lime for neutralization because it is a reagent more likely to be used as a neutralizing agent by a mine operator. The phosphate enclosure received a very small amount of super phosphate fertilizer. This amount was expected to cause a trophic status similar to that of Southern Illinois lakes that are not impacted by acid mine drainage. The sewage sludge and straw enclosures received loadings similar to those added in experiment 2 on 5/31/88. As usual, open lake samples were taken to control for enclosure effects.

Table 4: Experiment 3 - June 22 to August 17, 1988 - 9 weeks - 6m depth

	Date of Addition
Treatment	6/22
Control (C1)	Nothing
Control (C2)	Nothing
Lime	Hydrated Lime 1.32 kg
Phosphate	Super Phosphate Fertilizer .05 kg
Sewage (Sew)	Sludge 9.4 kg
Straw	Straw 4.2 kg
Open	Open lake measurements

Results - Experiment 3

Standing stocks of net ANC do not show any clear trends for Experiment 3 (Figs. 10 and 11). The values for the lime enclosure tend to be higher than the controls, but most other enclosures showed no higher ANC.

The Wilcoxon Signed Rank Test results (Table 5) show that only lime additions had major effects on water quality. Aluminum, iron, manganese and zinc concentrations all show statistically significant differences between lime and control enclosures.

Straw additions had little effect on water quality in Experiment 3. They neither reduced oxygen levels nor removed metal ions.

Sulfide levels remained low in all enclosures and the open lake, except in the bottom layers (Fig. 12). lime additions produced more sulfide than either of the organic matter additions.

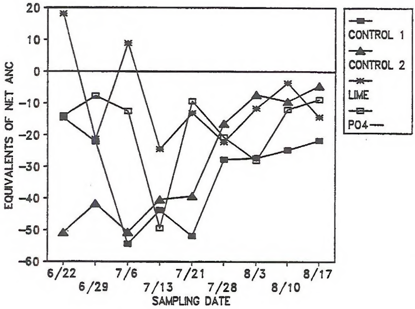


Figure 10: Standing Stock of Net Acid Neutralizing Capacity (ANC) in Experiment 3.

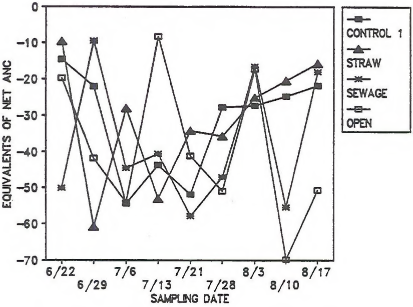


Figure 11: Standing Stock of Net Acid Neutralizing Capacity (ANC) in Experiment 3.

Table 5: Wilcoxon Signed Rank Test on Experiment #3. Z statistics are listed.
* .05<P<.1 **.01<P<.05 ***P<.01

Temperature

	C1	C2	Lime	PO4	Straw	Sewage	Open
C1		-1.7 *	-0.03	-2.94 ***	-0.77	-0.53	-1.37
C2			-2.26 **	-1.05	-1.89 *	-0.62	-2.99 ***
Lime				-3.15 ***	-0.85	-1.8 *	-1.1
PO4					-2.82 ***	-1.29	-3.38 ***
Straw						-0.18	-1.74 *
Sewage							-1.8 *
Open							

Sulfide

	C1	C2	Lime	PO4	Straw	Sewage	Open
C1		-1.21	-2.27 **	-0.37	-0.94	-0.37	-0.37
C2			-0.59	-1.36	-0.94	-0.94	-1.15
Lime				-3.05 ***	-2.35 **	-2.78 ***	-2.76 ***
PO4					-1.46	-0.45	-0.8
Straw						-1.34	-0.91
Sewage							0
Open							

Oxygen

	C1	C2	Lime	PO4	Straw	Sewage	Open
C1		-0.11	-0.18	-0.34	-0.03	-0.1	-1.17
C2			-0.76	-0.07	-0.23	-0.34	-1.53
Lime				-0.15	-0.14	-0.27	-1.14
PO4					-0.28	-0.04	-0.75
Straw						-0.14	-1.25
Sewage							
Open							

Iron

	C1	C2	Lime	PO4	Straw	Sewage	Open
C1		-2.61 ***	-2.22 **	-0.36	-3.98 ***	-0.24	-0.66
C2			-1.86 *	-1.44	-3.34 ***	-1.83 *	-1.52
Lime				-0.03	-3.57 ***	-0.65	3.94 ***
PO4					-2.37 **	-0.61	-1.5
Straw						-2.06 *	-0.76
Sewage							-1.11
Open							

pH

	C1	C2	Lime	PO4	Straw	Sewage	Open
C1		-0.82	-4.46 ***	-1.44	-3.01 ***	-1.46	-2.71 ***
C2			-4.35 ***	-0.38	-1.29	-0.24	-2.57 ***
Lime				-4.43 ***	-4.37 ***	-4.43 ***	-4.43 ***
PO4					-0.86	-0.89	-2.56 **
Straw						-1.57	-1.5
Sewage							-2.84 ***
Open							

Alkalinity

	C1	C2	Lime	PO4	Straw	Sewage	Open
C1		-1	-4.37 ***	-0.32	-1	-0.45	-0.45
C2			-4.29 ***	0	0	-1.34	-1
Lime				-4.37 ***	-4.37 ***	-4.37 ***	-3.92 ***
PO4					0	-1.34	-1
Straw						-1.34	-1
Sewage							0
Open							

Sulfate

	C1	C2	Lime	PO4	Straw	Sewage	Open
C1		-1.2	-0.9	-0.31	-0.55	-1.79	-0.9
C2			-1.97 **	-1.31	-2.71 ***	-3.05 ***	-1.41
Lime				-0.99	-0.18	-0.29	-0.22
PO4					-0.72	-1.25	-0.62
Straw						-0.59	-0.67
Sewage							-0.83
Open							

Acidity

	C1	C2	Lime	PO4	Straw	Sewage	Open
C1		-1.77 *	-3.82 ***	-1.14	-0.19	-0.34	-0.54
C2			-3.04 ***	-0.26	-1.76 *	-1.25	-0.05
Lime				-1.83 *	-3.41 ***	-3.33 ***	-3.91 ***
PO4					-2.77 ***	-1.74 *	-0.85
Straw						-0.22	-0.67
Sewage							-0.18
Open							

Table 5: Continued

Zinc

	C1	C2	Lime	PO4	Straw	Sewage	Open
C1		-2.03 **	-4.47 ***	-1.83 *	-0.19	-1.38	-2.1 **
C2			-3.89 ***	-0.53	-1.73 *	-0.9	-1.17
Lime				-4.47 ***	-4.4 ***	-4.31 ***	-4.41 ***
PO4					-1.26	-0.34	-0.93
Straw						-0.75	-2.55 *
Sewage							-0.81
Open							

Manganese

	C1	C2	Lime	PO4	Straw	Sewage	Open
C1		-2.12 *	-2.48 *	-2.88 ***	-0.05	-0.7	-0.62
C2			-1.98 **	-0.37	-2.06 **	-2.18 **	-0.49
Lime				-1.18	-2.19 **	-2.19 **	-3.11 ***
PO4					-2.74 ***	-2.49 *	-1.42
Straw						-0.86	-0.31
Sewage							-0.21
Open							

Total Phosphorus

	C1	C2	Lime	PO4	Straw	Sewage	Open
C1		-1.7 *	-0.55	-1.36	-0.34	-0.2	-1.03
C2			-1.97 **	-2.46 **	-0.69	-1.18	-0.27
Lime				-1.37	-1.14	-0.6	-1.63
PO4					-2.97 ***	-2.5 **	-2.22 **
Straw						-0.57	-1.36
Sewage							-0.34
Open							

Aluminum

	C1	C2	Lime	PO4	Straw	Sewage	Open
C1		-2.3 **	-4.42 ***	-2.68 ***	0	-0.13	-0.16
C2			-4.2 ***	-0.5	-2.07 **	-2.81 ***	-1.92 *
Lime				-4.28 ***	-4.35 ***	-4.43 ***	-3.92 ***
PO4					-2.4 **	-2.49 **	-0.82
Straw						-0.91	-1.82 *
Sewage							-1.9 *
Open							

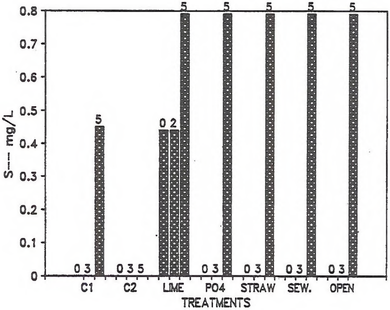


Figure 12: Comparisons of sulfide concentrations among treatments in Experiment 3 on 7/6/88. Numbers on columns are depths in the enclosures.

Discussion - Experiment 3

The results of experiment 3 were disappointing because little sulfate reduction occurred. The most sulfide production occurred in the lime enclosure where no organic matter had been added. As expected, additions of $Ca(OH)_2$ removed metal ions from the water column and raised alkalinity in addition to encouraging a small amount of sulfate reduction.

We propose three alternative hypotheses to explain the lack of sulfate reduction in Experiment 3. The first hypothesis is that, although we used enough straw to produce sulfate reduction in Experiment 2, we still were not providing enough for the deeper enclosures used in Experiment 3.

The second hypothesis is that low pH inhibits sulfate reducing bacteria. The results of Baas-Becking (4) and Schuurkes and Kok (32) support this hypothesis. Our discovery that the lime enclosure had the most sulfide production in Experiment 3 also suggests that this hypothesis might be true.

A third, less likely, hypothesis is that sulfate reducing bacteria were absent from our enclosures and that sulfate reduction did not occur. We knew that sulfate reduction was occurring in the sediment in some portions of the lake but sulfate-reducing bacteria might be excluded from our enclosures for some reason unknown to us.

Experiment 4 - 8/24/88 to 8/27/88

Purpose

The purpose of this experiment was to test the hypotheses generated by the results of experiment 3.

Additives - Experiment 4 (Table 6)

Because even the increased levels of organic matter used in experiment 3 failed to produce neutralization, in experiment 4 organic matter was added every week for the first three weeks until neutralization occurred (Table 6).

Two new enclosures replaced the phosphorus enclosure and one of the controls (Table 6). These new enclosures were used to test the effect of raised pH on sulfate reduction. Baas-Becking *et al*.(4) and Schuurkes and Kok (32) suggested that sulfate reduction was inhibited by low pH. The new enclosures received the same amounts of straw and sewage sludge as the straw and sewage enclosures, but they also received the same amount of limestone added to the lime enclosure. As with previous experiments, one enclosure was reserved as a controls. In addition, samples were taken from the open lake to control for enclosure effects.

Table 6: Experiment 4 - August 24 to September 27, 1988 - 6 weeks
6 m depth

	Date of Addition		
Treatment	8/24	8/31	9/7
Control (C)	Nothing	Nothing	Nothing
Lime	3.6 kg Limestone	1.8 kg Limestone	.9kg Hydrated Lime
Straw	10 kg Straw	5 kg Straw	5 kg Straw
Sewage (Sew)	20 kg Sludge	10 kg Sludge	10 kg Sludge
Lime+Straw (LStraw)	3.6 kg Limestone + 10 kg Straw	1.8 kg Limestone + 5 kg Straw	.9kg Hydrated Lime + 5 kg Straw
Lime+Sewage (LSew)	3.6 kg Limestone + 10 kg Sludge	1.8 kg Limestone + 5 kg Sludge	.9kg Hydrated Lime + 5 kg Sludge
Open	Open lake measurements		

Results - Experiment 4

In contrast to Experiment 3, straw additions produced major changes in water chemistry during Experiment 4. As expected, standing stocks of net ANC increased in all enclosures to which $Ca(OH)_2$ was added (Fig 13). It also increased in the straw enclosure (Fig. 14), but the largest increase occurred where a combination of lime and straw were added (Fig 13).

The Wilcoxon Signed Rank Test shows the strong chemical differentiation among enclosures during experiment 4 (Table 7). Throughout most of the experiment, oxygen was absent in straw enclosures (Fig. 15) but present in other enclosures. Sulfide levels were high in the two enclosures that were dosed with straw (Figs. 16 and 17) but not in others. The sulfide levels in the lime/straw enclosure were higher than in the straw enclosure. Sulfide was absent in the sewage enclosures. The pH rose to 8.0 in the straw enclosure even without the addition of $Ca(OH)_2$ (Fig. 18). In the straw enclosure, alkalinity increased and acidity declined to 0 (Figs. 19 and 20). In contrast with sulfide, sulfate concentrations were unaffected by straw additions (Figs. 21 and 22). Iron concentrations were a little higher in the straw enclosures than in the control enclosure (Fig. 23 and 24, Table 7). Although lime additions reduced iron concentrations in the deep water of the lime enclosures (Fig. 25), the concentrations were not significantly different from those in the other enclosures (Table 7). In general, the deeper waters of the lime and sewage enclosures accumulated high concentrations of iron (Fig. 26).

Aluminum was least affected by straw additions. It declined where $Ca(OH)_2$ was added and actually increased in the presence of

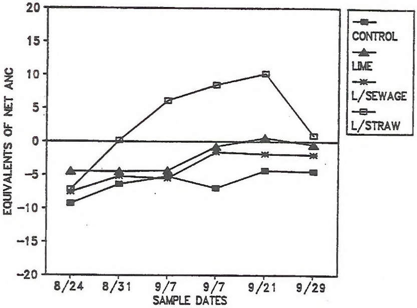


Figure 13: Standing Stock of Net Acid Neutralizing Capacity (ANC) in Experiment 4.

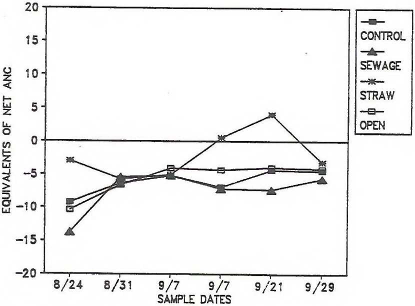


Figure 14: Standing Stock of Net Acid Neutralizing Capacity (ANC) in Experiment 4.

Table 7: Wilcoxon Signed Rank Test on Experiment #4. Z statistics are listed.

* .05<P<.1 **.01<P<.05 ***P<.01

Temperature

	Contr.	Lime	L/Sew	L/Straw	Sewage	Straw	Open
Contr.		-2.44 **	-0.59	-0.59	-0.02	-0.38	-0.59
Lime			-0.84	-0.92	-0.94	-1.33	-1.88
L/Sew				-0.91	-0.36	-0.42	-1.13
L/Straw					0	-1.11	-1.63
Sewage						-0.04	-0.59
Straw							-0.14
Open							

Sulfide

	Contr.	Lime	L/Sew	L/Straw	Sewage	Straw	Open
Contr.		-1.46	-1.46	-3.18 ***	-2.03 **	-3.06 ***	0
Lime			-0.14	-3.1 ***	-0.91	-2.97 ***	-1.6
L/Sew				-3.18 ***	-0.52	-2.82 ***	-1.6
L/Straw					-2.98 ***	-2.41 **	-3.18 ***
Sewage						-2.76 ***	-1.78 **
Straw							-3.06 ***
Open							

Oxygen

	Contr.	Lime	L/Sew	L/Straw	Sewage	Straw	Open
Contr.		-2.3 **	-2.07 **	-3.72 ***	-3.16 ***	-3.72 ***	-0.02
Lime			-0.85	-3.12 ***	-0.5	-3.2 **	-2.59 ***
L/Sew				-3.1 ***	-0.11	-3.29 ***	-2.68 ***
L/Straw					-3.34 ***	-1.24	-3.59 ***
Sewage						-3.57 ***	-2.9 ***
Straw							-3.72 ***
Open							

Iron

	Contr.	Lime	L/Sew	L/Straw	Sewage	Straw	Open
Contr.		-0.05	-0.19	-0.46	-0.72	-2.44 **	-2.6 ***
Lime			-0.73	-0.32	-1.16	-0.02	-0.37
L/Sew				-0.15	-2.24 **	-1.11	-1.37
L/Straw					-0.45	-0.41	-1.66 *
Sewage						-0.07	-0.33
Straw							-1.44
Open							

pH

	Contr.	Lime	L/Sew	L/Straw	Sewage	Straw	Open
Contr.		-3.72 ***	-3.21 ***	-3.72 ***	-0.22	-3.64 ***	0.1
Lime			-3.37 ***	-2.08 **	-3.62 ***	-2.72 ***	-3.72 ***
L/Sew				-3.72 ***	-3.53 ***	-2.26 **	-3.27 ***
L/Straw					-3.62 ***	-3.22 ***	-3.72 ***
Sewage						-3.62 ***	-1.26
Straw							-3.72 ***
Open							

Alkalinity

	Contr.	Lime	L/Sew	L/Straw	Sewage	Straw	Open
Contr.		-3.29 ***	-1.34	-3.62 ***	0	-2.36 **	0
Lime			-3.29 ***	-3.53 ***	-3.29 ***	-0.28	-3.29 ***
L/Sew				-3.62 ***	-1.34	-2.37	-1.34
L/Straw					-3.62 ***	-3.62 ***	-3.62 ***
Sewage						-2.36 **	0
Straw							-2.37 **
Open							

Sulfate

	Contr.	Lime	L/Sew	L/Straw	Sewage	Straw	Open
Contr.		-0.43	-0.11	-0.21	-0.31	-1.37	-0.06
Lime			-1.02	-0.85	-0.52	-1.68	-0.46
L/Sew				-0.43	-0.17	-0.88	-1.07
L/Straw					-0.36	-1.61	-1.04
Sewage						-1.37	-0.37
Straw							-1.53
Open							

Acidity

	Contr.	Lime	L/Sew	L/Straw	Sewage	Straw	Open
Contr.		-2.95 ***	-2.9 ***	-3.52 ***	-0.7	-3.1 ***	-1.98 **
Lime			-1.36	-1.45	-2.69 ***	-0.54	-2.28 **
L/Sew				-2.86 ***	-2.96 ***	-3.55 ***	-2.85 ***
L/Straw					-3.57 ***	-2.59 ***	-3.57 ***
Sewage						-2.97 ***	-2.5 **
Straw							-2.45 **
Open							

Table 7: Continued

Zinc

	Contr.	Lime	L/Sew	L/Straw	Sewage	Straw	Open
Contr.		-2.58 ***	-1.22	-3.68 ***	-0.92	-3.24 ***	-0.15
Lime			-2.68 ***	-2.35 **	-1.63	-3.51 ***	-2.03 **
L/Sew				-3.72 ***	-0.76	-3.2 ***	-0.31
L/Straw					-3.36 ***	-3.62 ***	-3.42 ***
Sewage						-2.25 **	-0.98
Straw							-1.57
Open							

Manganese

	Contr.	Lime	L/Sew	L/Straw	Sewage	Straw	Open
Contr.		-0.43	-1.62 *	-2.76 ***	-2.66 ***	-3.59 ***	-3.44 ***
Lime			-1.55	-1.16	-1.66 *	-2.75 ***	-1.57
L/Sew				-0.11	-0.44	-3.31 ***	-1.35
L/Straw					-0.26	-2.29 **	-0.66
Sewage						-1.89 *	-0.74
Straw							-2.34 **
Open							

TOTAL PHOSPHORUS

	Contr.	Lime	L/Sew	L/Straw	Sewage	Straw	Open
Contr.		-0.74	-2.72 ***	-2.84 ***	-3.05 ***	-3.12 ***	-1.78 *
Lime			-2.86 ***	-3.31 ***	-3.52 ***	-3.41 ***	-0.28
L/Sew				-1.76 *	-2.16 **	-1.92 *	-3.38 ***
L/Straw					-1.56	-3.05 ***	-3.21 ***
Sewage						-0.97	-3.26 ***
Straw							-3.35 ***
Open							

Aluminum

	Contr.	Lime	L/Sew	L/Straw	Sewage	Straw	Open
Contr.		-3.72 ***	-2.28 **	-3.68 ***	-3.72 ***	-2.55 **	-0.72
Lime			-3.64 ***	-3.03 ***	-3.72 ***	-3.64 ***	-3.72 ***
L/Sew				-3.46 ***	-3.36 ***	-0.63	-2.68 ***
L/Straw					-3.72 ***	-2.94	-3.72 ***
Sewage						-3.72 ***	-3.44 ***
Straw							-2.8 ***
Open							

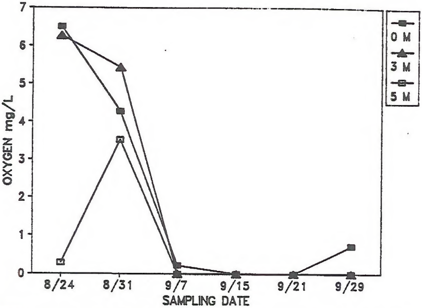


Figure 15: Variations in oxygen concentrations at 3 depths in the straw enclosure during Experiment 4.

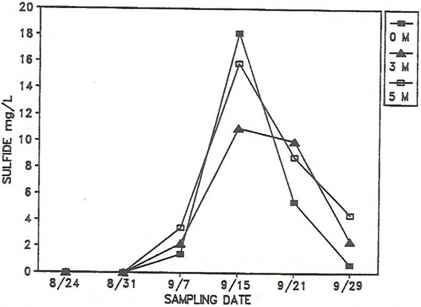


Figure 16: Variations in sulfide concentrations at 3 depths in the straw enclosure during Experiment 4.

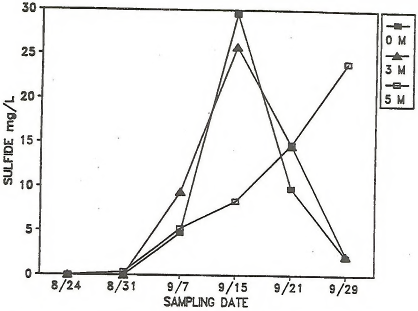


Figure 17: Variations in sulfide concentrations at 3 depths in the Lime/Straw enclosure during Experiment 4.

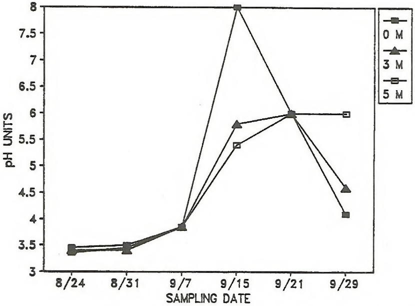


Figure 18: Variations in pH at 3 depths in the Straw enclosures during Experiment 4.

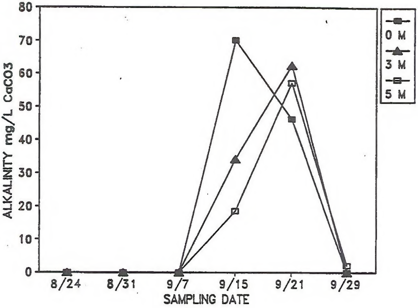


Figure 19: Variations in alkalinity at 3 depths in the straw enclosure during Experiment 4.

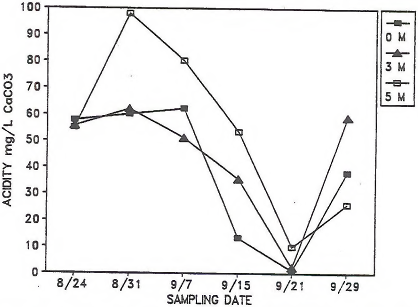


Figure 20: Variations in acidity at 3 depths in the straw enclosure during experiment 4.

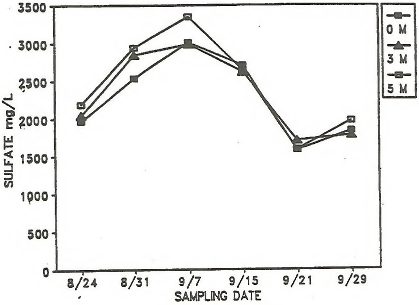


Figure 21: Variations in sulfate at 3 depths in the Lime/Straw enclosure during Experiment 4

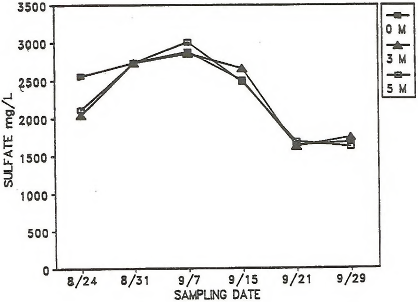


Figure 22: Variations in sulfate at 3 depths in the Straw enclosure during experiment 4.

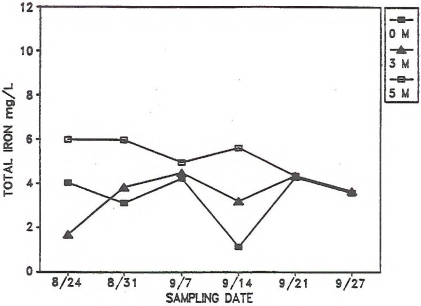


Figure 23: Variations in iron at 3 depths in the Straw enclosure during Experiment 4.

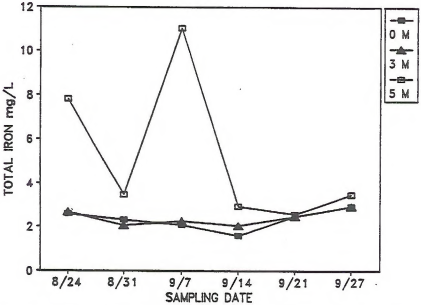


Figure 24: Variations in iron concentrations in the control enclosure during Experiment 4.

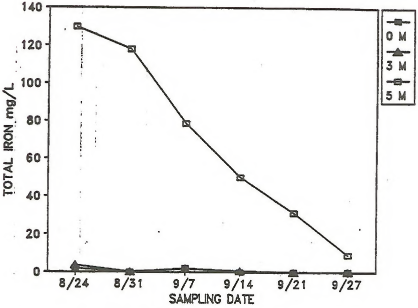


Figure 25: Variations in iron concentrations at 3 depths in the straw enclosure during Experiment 4.

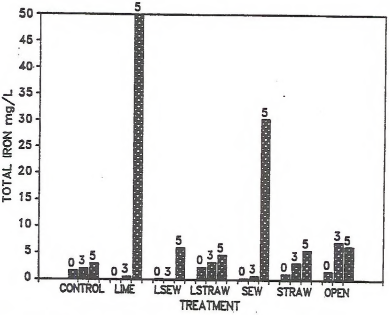


Figure 26: Comparisons of iron concentrations between treatments in Experiment 4 on 9/15/88. Numbers on columns are depths in enclosures.

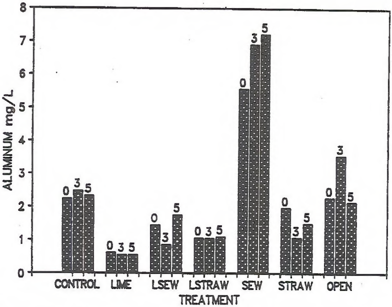


Figure 27: Comparisons of aluminum concentrations between treatments in Experiment 4 on 9/15/88. Numbers on columns are depths in enclosures.

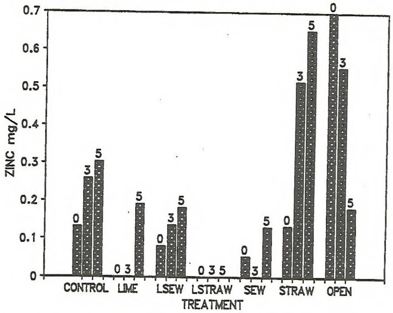


Figure 28: Comparisons among zinc concentrations in Experiment 4 on 9/15/88 Numbers on columns represent depths in enclosures.

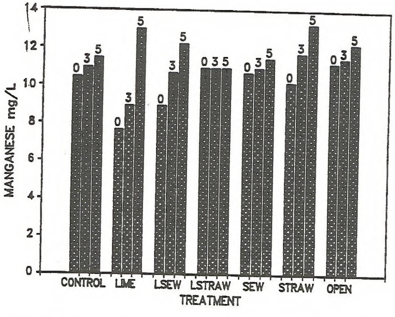


Figure 29: Comparisons of manganese concentrations among treatments in Experiment 4 on 9/15/88. Numbers on columns represent depths in experimental enclosures.

sewage sludge (Fig. 27). Zinc was reduced by lime additions, but seemed to be increased by straw (Fig. 28). The high levels of aluminum in the straw enclosures approached those of the open lake. Manganese was unaffected by any treatment (Fig. 29).

Because we expected that the organic loading would increase trophic status of the test enclosures, we examined algal abundances during experiment 4. The species diversity of phytoplankton in the experimental enclosures and in the open lake was very low. The major species encountered were Chlamydomonas acidophila and Cryptomonas erosa and Chlorella. These have been found by other investigators in midwestern acid coal mine lakes (12). As the straw enclosures increased in sulfide, these species were replaced by Schroederia setigera.

The presence of sulfate-reducing bacteria was tested in water from the open lake and from each enclosure at the end of experiment 4. Sediment from the open lake and all enclosures except those dosed with straw were also tested for sulfate-reducing bacteria at the same time. Straw enclosures were excluded because the straw on the lake sediment prevented us from taking an adequate sediment sample.

The API Sulfate Broth Test showed that sulfate-reducers were present in the water and sediment of all tested enclosures. The bacteria were also present in the open lake sediment but not in the open lake water.

Discussion - Experiment 4

To test the hypotheses generated by Experiment 3, we changed the experimental design for Experiment 4. We discontinued the phosphate enclosure and one of the control enclosures. For Experiment 4 we constructed pairs of organically loaded enclosures, one with lime and the other without lime. The resulting combinations of additives were straw, lime/straw, sewage, and lime/sewage. An enclosure with lime only and a control enclosure were used to control for liming effects and for enclosure effects. These combinations were designed to test the hypothesis that low pH inhibits sulfate reducing bacteria. If such inhibition is important, then sulfide levels would be higher in enclosures with lime and organic matter than in those with organic matter alone.

We eliminated the possibility that our organic matter addition rates were too low by adding large amounts of material at weekly intervals until sulfide was produced. Small amounts of sulfide became detectable by the end of the second week of the experiment (September 7).

Sulfate reduction occurred in all enclosures that received straw additions. No sulfide appeared in the sewage enclosures. Apparently, as in experiment 2, the sewage addition was too stable to support sulfate reducing bacteria.

For sulfate reduction to occur conditions must be anaerobic. The oxygen concentrations in the straw enclosures declined to 0 as sulfate reduction began. As expected, the appearance of sulfide was accompanied by increases in pH and alkalinity. The pH rose to approximately the level expected if H_2S was functioning as a buffer (the pK_a for the first dissociation of H_2S is 6.95). The sulfide present in the water column also produced alkalinity. The raised pH also caused the precipitation of aluminum. Zinc levels declined, probably as a result of the production of zinc sulfide.

A few consequences of the sulfide production that we expected did not occur. There was no large loss of iron or manganese from the water column. Where there are high concentrations of sulfide one would expect the precipitation of Fe^{++} as sulfide. High pH should remove Mn^{++} from solution. Our iron and manganese analyses show no evidence of this loss. When samples were removed from the enclosures, however, a black precipitate formed in about 30 minutes. Stahl (36) noted a similar precipitation in his studies of an organically loaded alkaline surface mine lake. It appears that Fe^{++} and Mn^{++} are held in solution by some process that we don't yet understand.

Sulfate concentrations also remained unchanged by the additions of straw even when sulfide levels were quite high. When the molar concentrations of S^{--} and SO_4^{--} are examined it is clear that even at high sulfide levels (30 mg/l = .93 mmolar) the amount of sulfide is only a fraction of the sulfate (2,000 mg/l = 20.8 mmolar). Only about 5% of the standing stock of sulfate is reduced to sulfide. Our experimental error in the determination of sulfate is ± 24% (Table 1) so it is unlikely that we would be able to detect the loss of the small amount of sulfate that is reduced to sulfide.

Our tests for the presence of sulfate-reducing bacteria showed that all enclosures and all lake sediment had sulfate-reducers. Only the open lake water lacked them. Thus sulfate reduction is unlikely to be limited by the lack of organisms.

Our counts of phytoplankton in experiment 4 showed that there was no evidence of algal blooms caused by organic additions. The open lake and the control enclosure contained a species-poor phytoplankton community indicative of acid conditions. It included species found in other midwestern surface mine lakes (12). In the enclosures that received straw additions, *Schroderia setigera* soon replaced the acidophiles. *Schroederia* appears to be resistant to the toxic effects of H_2S. In summary, there is no evidence that organic treatments produce algal blooms.

The addition of lime to the straw enclosures seemed to enhance sulfate reduction as Schuurkes and Kok (32) suggested.

The lime/straw enclosure had higher sulfide levels than the straw enclosure and higher alkalinity than the lime enclosure. Thus, sulfate reducing bacteria were probably inhibited by low pH.

If sulfate reduction is limited by low pH, how can sulfate reduction occur in the straw enclosure where no lime was added? Sulfate reduction could continue at low rates under acidic conditions in the straw enclosure until the accumulation of H_2S raises pH enough to allow faster reduction rates. Liming may allow the higher rates to prevail from the beginning.

An alternative explanation for the higher sulfide levels in the lime-dosed enclosures is that the higher pH kept more of the sulfide in solution as HS^- and $S^=$. The lower pH of the enclosures without lime would allow more sulfide to remain as H_2S. The H_2S could outgas from the enclosure. At this time we have no way to differentiate between the hypothesis that higher pH caused higher rates of sulfide production and the hypothesis that it caused better retention of sulfide in the water column.

Experiment 5 - 4/4/89 to 6/13/89

Purpose

Because of the success of experiment 4 in demonstrating sulfate reduction, we hypothesized that improvements in neutralization might occur if the organic matter (straw) were trapped near the sediment/water interface at the bottom of the enclosures. We tested our hypothesis by sinking mesh bags of straw to the bottom of the test enclosures.

Additives - Experiment 5 (Table 8)

Organic matter additions to experiment 5 consisted of 9 kg quantities of straw. In deep straw and deep lime straw enclosures, the straw was added in mesh bags which were weighted to sink to the bottom of the enclosures. Unfortunately, because of the bouyancy of straw, the bags did not sink until the second week of the experiment (4/11). The bags were used to test whether results could be improved by trapping straw close to the sediment/water interface where dissimilatory sulfate reduction is expected to be vigorous.

Straw was added directly to the straw and lime straw enclosures without mesh bags. This straw floated in the enclosures until 4/11. It then sank to produce a 1 m thick layer of decomposing straw at the bottoms of the enclosures.

Lime, lime straw, and deep lime straw enclosures all received $CaCO_3$ treatments as 1.3 kg of chalk mixed with .7 kg of agricultural lime. These additions were done to test whether circumneutral pH was necessary to produce maximal rates of dissimilatory sulfate reduction.

Table 8: Experiment #5 - April 4 to June 13, 1989 - 10 weeks - 3 m depth

	Date of Addition	
Treatment	4/4	4/11
Control	Nothing	Nothing
Lime	Nothing	1.3 kg Chalk + .7 kg Limestone
Straw	9 kg Straw	Nothing
Lime/Straw	9 kg Straw	1.3 kg Chalk + .7 kg Limestone
Deep Straw	9 kg Straw in a mesh bag at bottom of enclosure	Nothing
Deep Lime/Straw	9 kg Straw in a mesh bag at bottom of enclosure	1.3 kg Chalk + .7 kg Limestone
Open Lake	Open Lake measurements	

Results - Experiment 5

When the plot of standing stock of net ANC for Experiment 5 is examined, it is apparent that every treatment in experiment 5 generated acid neutralizing capacity (Figs. 30 and 31). The most ANC was generated by the deep lime/straw treatment in which straw was held near the sediment surface and a mixture of chalk and limestone was applied to the enclosure.

When the Wilcoxon Signed Rank Test results are examined, the chemical differentiation among treatments becomes clearer (Table 9). Sulfide appeared in all straw-dosed enclosures (Figs. 32 and 33). A small amount of sulfide was also present in the bottom layers of the control enclosure and in the open lake (Figs. 34 and 35). This bottom layer was anaerobic and high in dissolved solids.

The pH increased in all enclosures, but pH in lime and lime/straw enclosures were not significantly different from one another (Table 9 and Fig. 36). Alkalinity rose in all treatment enclosures but remained 0 in the open lake and in the control enclosure (Fig. 37). Acidity declined and disappeared entirely in all of the lime-dosed enclosures and in the straw enclosure (Figs. 38 to 42). Acidity remained unchanged in the open lake (Fig. 43). As with the other experiments, sulfate levels were mostly unchanged by the treatments of experiment 5 (Fig. 44). The deep layers of the enclosures were higher in sulfate and aluminum than the more shallow layers (Fig. 44 and 45). Aluminum declined in all enclosures in which pH rose.

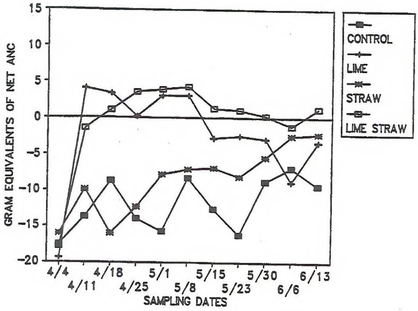


Figure 30: Standing Stock of Net Acid Neutralizing Capacity (ANC) in Experiment 5.

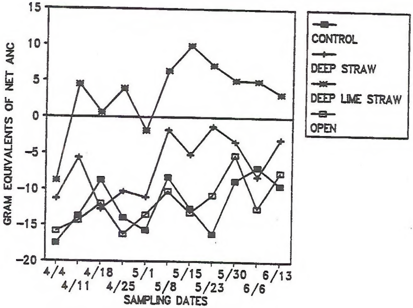


Figure 31 : Standing Stock of Net Acid Neutralizing Capacity (ANC) in Experiment 5.

Table 9: Wilcoxon Signed Rank Test on Experiment #5. Z statistics are listed.
* .D5<P<.1 **.01<P<.D5 ***P<.01

Temperature

	Contr.	Lime	Straw	LStraw	DeepStraw	DLStraw	Open
Contr.		-1	-1.69 *	-1.76 *	-0.11	-0.44	-0.37
Lime			-0.28	-0.75	-1.11	-1.23	-2.03 **
Straw				-1.38	-1.83	-1.2	-2.02 **
Lstraw					-2.51 **	-1.97 **	-2.22 **
DeepStraw						-0.19	-1.33
OLStraw							-0.89
Open							

pH

	Contr.	Lime	Straw	LStraw	DeepStraw	OLStraw	Open
Contr.		-5.01 ***	-4.89 ***	-5.01 ***	-4.99 ***	-4.99 ***	-2.93 ***
Lime			-2.56 **	-1.13	-4.3 ***	-3.58 ***	-4.72 ***
Straw				-3.25 ***	-2.7 ***	-4.85 ***	-4.64 ***
Lstraw					-4.88 ***	-3.85 ***	-4.96 ***
DeepStraw						-4.95 ***	-4.29 ***
DLStraw							-4.99 ***
Open							

Sulfide

	Contr.	Lime	Straw	LStraw	DeepStraw	DLStraw	Dpen
Contr.		-0.15	-4.3 ***	-3.21 ***	-2.42 **	-4.56 ***	-1.36
Lime			-4.22 ***	-3.25 ***	-3.01 ***	-4.4 ***	-2.27 **
Straw				-2.98 ***	-2.98 ***	-1.65 *	-4.32 ***
Lstraw					-1.37	-2.57 **	-3.22 ***
DeepStraw						-3.55 ***	-1.61
DLStraw							-4.75 ***
Dpen							

Alkalinity

	Contr.	Lime	Straw	LStraw	DeepStraw	DLStraw	Open
Contr.		-4.2 ***	-4.01 ***	-4.37 ***	-2.52 **	-4.78 ***	0
Lime			-2.27 **	-2.66 ***	-4.01 ***	-4.66 ***	-4.2 ***
Straw				-3.54 ***	-3.68 ***	-4.78 ***	-4.01 ***
Lstraw					-4.37 ***	-3.51 ***	-4.37 ***
DeepStraw						-4.78 ***	-2.52 **
DLStraw							-4.78 ***
Dpen							

Oxygen

	Contr.	Lime	Straw	LStraw	DeepStraw	DLStraw	Open
Contr.		-0.75	-4.25 ***	-3.57 ***	-3.89 ***	-4.32 ***	-0.59
Lime			-4.32 ***	-3.92 ***	-4.25 ***	-4.38 ***	-1.69 *
Straw				-279 ***	-3.32 ***	-1.58	-4.63 ***
Lstraw					-0.56	-2.93 ***	-3.95 ***
DeepStraw						-3.66 ***	-4.13 ***
DLStraw							-4.49 ***
Open							

Sulfate

	Contr.	Lime	Straw	LStraw	DeepStraw	OLStraw	Dpen
Contr.		-0.17	-1.48	-1.33	-1.27	-2.53 **	-2.23 **
Lime			-1.62	-1.6	-1.21	-3.06 ***	-1.88 *
Straw				-0.11	-1.33	-1.94 *	-2.87 ***
Lstraw					-1.08	-2.36 **	-2.7 ***
DeepStraw						-2.52 **	-2.08 **
DLStraw							-3.59 ***
Dpen							

Iron

	Contr.	Lime	Straw	LStraw	OeepStraw	OLStraw	Open
Contr.		-4.01 ***	-1.1	-0.51	-0.79	-0.9	-1.79
Lime			-3.37 ***	-1.59	-1.89 *	-1.19	-4.89 ***
Straw				-3.37 ***	-2.96 ***	-4.23 ***	-0.75
Lstraw					-D.46	-D.49	-1.4
OeepStraw						-0.61	-2.21 **
DLStraw							-0.72
Open							

Acidity

	Contr.	Lime	Straw	LStraw	OeepStraw	DLStraw	Dpen
Contr.		-4.23 ***	-1.87 *	-4.49 ***	-3.44 ***	-4.99 ***	-1.00
Lime			-3.55 ***	0	-2.84 **	-1.63 ***	-4.22 ***
Straw				-4.11 ***	-3.1 ***	-4.57 ***	-0.41
Lstraw					-3.D5 ***	-2.28 **	-3.82 ***
OeepStraw						-4.51 ***	-2.92 ***
DLStraw							-4.99 ***
Open							

Table 9: Contiued

Zinc

	Contr.	Lime	Straw	LStraw	DeepStraw	DLStraw	Open
Contr.		-4.6 ***	-4.17 ***	-5.01 ***	-4.64 ***	-4.76 ***	-2.14 **
Lime			-0.8	-3.87 ***	-0.82	-2.74 ***	-3.22 ***
Straw				-3.2 ***	-0.73	-3.28 ***	-2.4 **
Lstraw					-4.15 ***	-1.14	-4.61 ***
DeepStraw						-4.52 ***	-2.03 **
DLStraw							-4.01 ***
Open							

Manganese

	Contr.	Lime	Straw	LStraw	DeepStraw	DLStraw	Open
Contr.		-0.92	-0.24	-2.77 ***	-2.27 **	-1.66 *	-0.14
Lime			-1.15	-1.54	-2.64 ***	-1.8 *	-0.54
Straw				-2.74 ***	-2.39 **	-3.99 ***	-0.17
Lstraw					-0.39	-0.67	-1.22
DeepStraw						-1.05	-1.01
DLStraw							-0.69
Open							

Cadmium

	Contr.	Lime	Straw	LStraw	DeepStraw	DLStraw	Open
Contr.		-1.37	-2.14 **	-0.43	-0.25	-1.53	-1.74 *
Lime			-0.49	-0.93	-2.16 **	-0.28	-0.15
Straw				-1.47	-1.59	-0.11	-0.21
Lstraw					-0.27	-1.00	-0.84
DeepStraw						-1.41	-0.72
DLStraw							-0.36
Open							

Aluminum

	Contr.	Lime	Straw	LStraw	DeepStraw	DLStraw	Open
Contr.		-4.95 ***	-4.42 ***	-4.6 ***	-4.71 ***	-5.01 ***	-0.85
Lime			-3.48 ***	-0.49	-4.19 ***	-0.65	-4.87 ***
Straw				-4.24 ***	-0.94	-4.44 ***	-4.1 ***
Lstraw					-4.46 ***	-1.03	-4.6 ***
DeepStraw						-4.94 ***	-3.21 ***
DLStraw							-4.94 ***
Open							

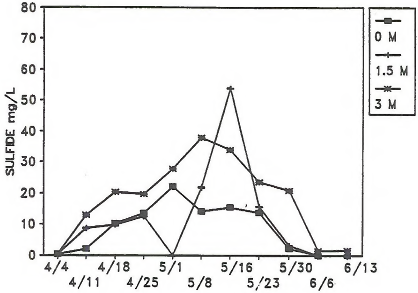


Figure 32: Variations in sulfide concentrations at 3 depths in the straw enclosure during experiment 5.

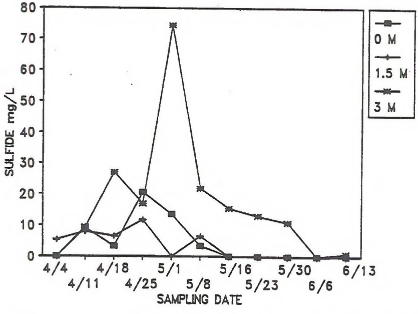


Figure 33: Variations in sulfide concentrations at 3 depths in the Lime/Straw enclosure during Experiment 5.

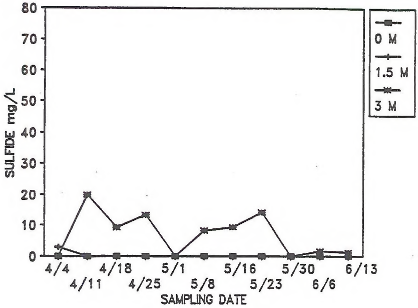


Figure 34: Variations in sulfide concentrations in the control enclosure during Experiment 5.

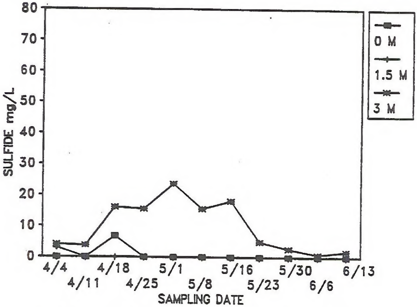


Figure 35: Variations in sulfide concentrations in the open lake during Experiment 5.

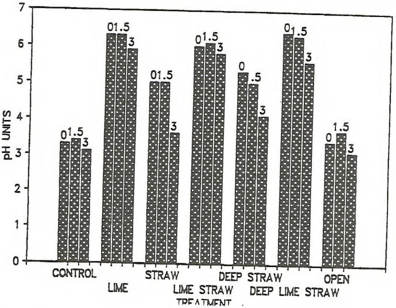


Figure 36: Comparisons of pH among treatments in Experiment 5 on 4/18/89. Numbers on columns are depths in enclosures.

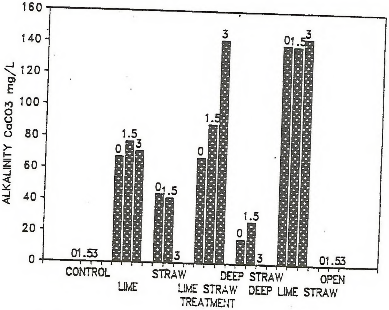


Figure 37: Comparisons of alkalinity among treatments in Experiment 5 on 4/18/89. Numbers on columns are depths in enclosures.

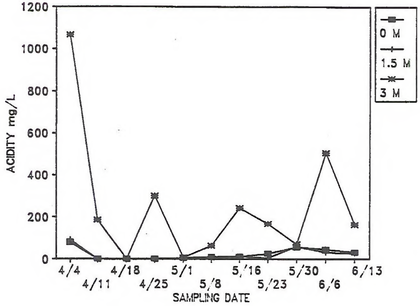


Figure 38: Variations in acidity at 3 depths in the Lime enclosure during Experiment 5.

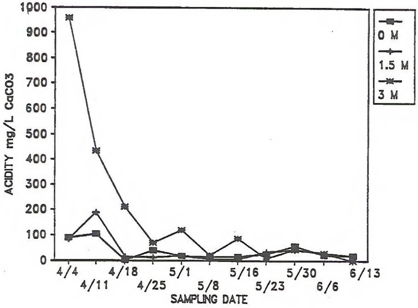


Figure 39: Variations in acidity at 3 depths in the lime/straw enclosure during experiment 5.

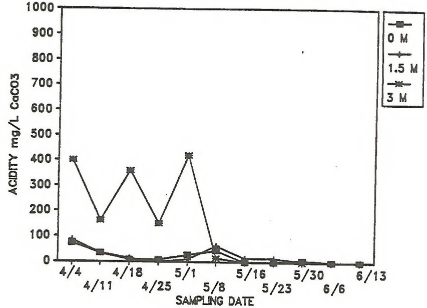


Figure 40: Variations in acidity at 3 depths in the deep lime/straw enclosure during experiment 5.

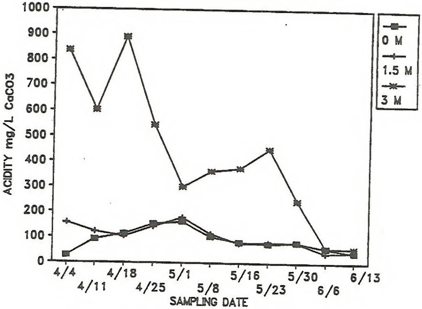


Figure 41: Variations in acidity at 3 depths in the straw enclosure during Experiment 5.

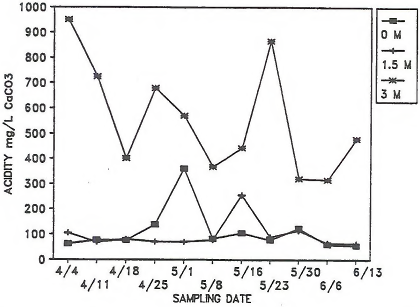


Figure 42: Variations in acidity of the control enclosure during Experiment 5.

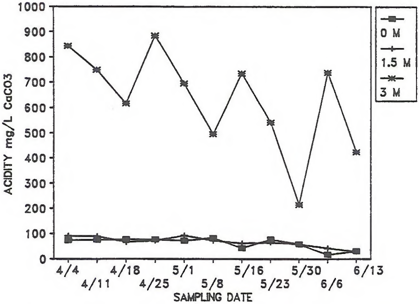


Figure 43: Variations in the acidity of the open lake during Experiment 5.

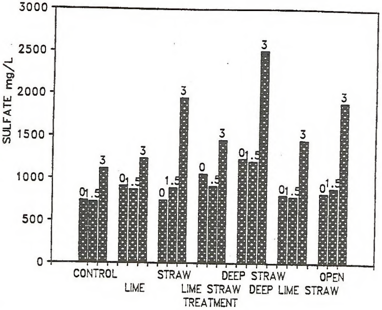


Figure 44: Comparisons of sulfate concentrations among treatments in Experiment 5 on 4/18/89. Numbers on columns are depths in the enclosure.

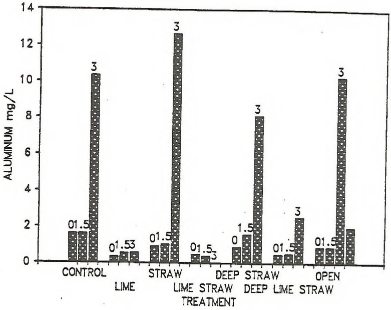


Figure 45: Comparisons of aluminum concentrations among treatments in Experiment 5 on 4/18/89. Numbers on columns are depths in the enclosures.

As in experiment 4, iron concentrations were greater than 100 mg/l in the deepest parts of the enclosures (Fig. 46). For the sake of clarity, these levels will not be depicted on the graphs of iron concentration (Figs. 47 to 50). Lime additions caused sharp declines of iron (Fig. 47) which made the concentrations significantly lower in the lime enclosures than in the control and straw enclosures (Table 9). Control concentrations also declined (Fig. 48), but not as much as in the lime enclosure (Fig. 47). Straw additions actually increased iron concentrations (Fig. 49), but the difference between the straw enclosure and the control was not significant (Table 9). The lime/straw enclosure (Fig. 49) had an iron concentration intermediate between the control and lime enclosures. It was not significantly different from either of these. (Table 9).

Secchi disc depths were very shallow (.5 m or less) in enclosures dosed with straw (Figs 51 and 52). These enclosures had black, extremely turbid water (probably with some FeS - in suspension). The control and lime enclosures and the open lake had deeper Secchi disc depths (1 to 2 m).

Discussion - Experiment 5

In this experiment we tested straw as a source of organic matter for the neutralization process. Because the lake sediment is likely to be the site of sulfate reduction, we tested whether neutralization might be enhanced if we kept the straw at the bottom of the enclosure near the sediment/water interface.

We also lengthened the experiment to determine if the neutralization produced by organic additions could persist.

As in Experiment 4, sulfate reduction occurred in all enclosures that were treated with straw. The results were similar. Sulfate reduction occurred where oxygen levels were low. Reduction of sulfate caused increases in pH and in alkalinity. Aluminum declined but iron and manganese did not.

Experiment 5 shows even more clearly that a combination of lime and straw is more likely to result in long-term neutralization. The deep lime/straw enclosure had acid neutralizing capacity for the whole period of the experiment. Unfortunately, the enclosure was also anaerobic for the whole time. This result means that although the organic additions can cause neutralization, they also make the water column uninhabitable for multicellular organisms.

Experiment 6 - 7/20/89 to 9/27/89

Purpose

In experiment 6 we tried to test different loading rates to

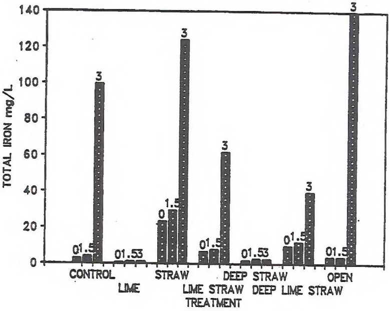


Figure 46: Comparisons of iron concentrations among treatments in Experiment 5 on 5/9/89. Numbers on columns represent the sampling depths in the experimental enclosures.

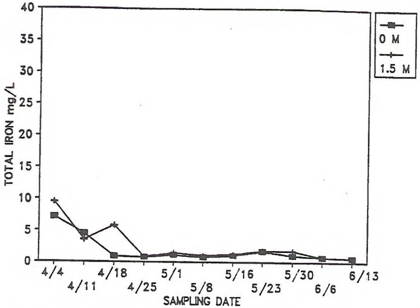


Figure 47: Variations in iron concentrations at 2 depths in the lime enclosure during Experiment 5.

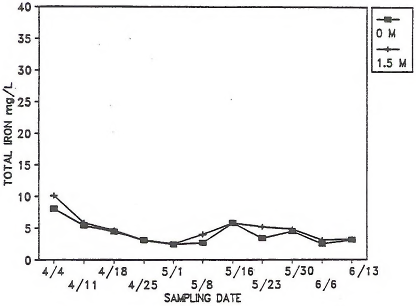


Figure 48: Variations in iron concentrations at 2 depths in the control enclosure during Experiment 6.

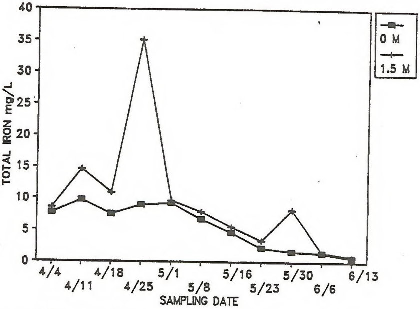


Figure 49: Variations in iron concentrations at 2 depths in the Lime/Straw enclosure during Experiment 5.

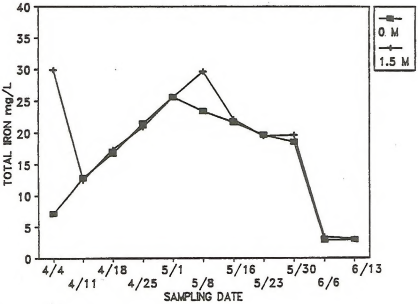


Figure 50: Variations in iron concentrations at 2 depths in the straw enclosure during Experiment 5.

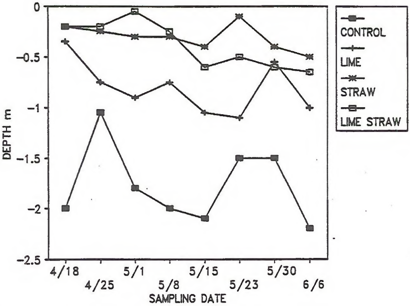


Figure 51 : Variations in Secchi disc transparency in experimental enclosures during Experiment 5.

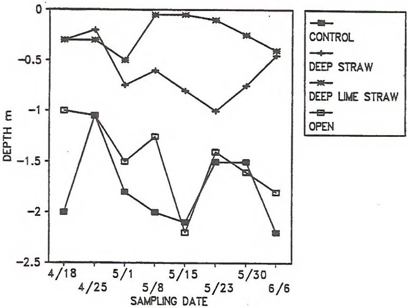


Figure 52: Variations in Secchi disc transparency in experimental enclosures during Experiment 5.

determine how neutralization varied with different amounts of organic matter additive.

Additives - Experiment 6 (Table 10)

In experiment 6 we added different amounts of wheat straw to the enclosures as a source of organic matter. One enclosure was reserved as a control and had no organic additives. Straw was added as fractions of a 15 kg bale. The amount of straw added to each enclosure is summarized in Table 10.

Table 10: Experiment #6 - July 20, to September 27, 1989 - 10 weeks
3m depth

Treatment	Date of Addition 7/20
Control	Nothing added
3/4	3/4 bale - 11.25 Kg straw
1/2	1/2 bale - 7.50 Kg straw
3/8	3/8 bale - 5.63 Kg straw
1/4	1/4 bale - 3.75 Kg straw
1/8	1/8 bale - 1.88 Kg straw
Open Lake	Open Lake measurements

Results - Experiment 6

The purpose of experiment 6 was to test how different organic matter loading rates would affect the neutralization process. Different loading rates resulted in different amounts of ANC being generated in each enclosure. Higher loading rates resulted in more ANC generation and longer durations of neutralization (Figs. 53 and 54). The 3/4 bale enclosure was an exception to this observation. At the end of the experiment we learned that this enclosure had been improperly installed so that the bottom was open to the lake rather than being sunk in the mud. Results from this enclosure are, therefore, suspect. Enclosures with low organic matter dosage (1/4 and 1/8 bale) showed no generation of acid neutralizing capacity.

The Wilcoxon Signed Rank Test shows the differentiation among enclosures that results from different organic matter loading rates. A few ions, like iron, manganese and sulfate remained unchanged by any treatment (Table 11).

In contrast, a number of other chemical variables changed along with ANC. In the enclosures receiving the heaviest organic matter loadings, oxygen and acidity declined (Figs. 55 and 56) as sulfide, pH, and alkalinity rose (Figs. 57 to 59). Among the metal ions, only aluminum and zinc declined (Figs. 60 and 61). It is not clear why the measurements were elevated in the control enclosure (Fig. 61).

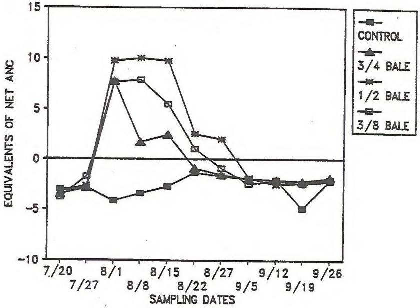


Figure 53: Standing Stock of Net Acid Neutralizing Capacity (ANC) in Experiment 6.

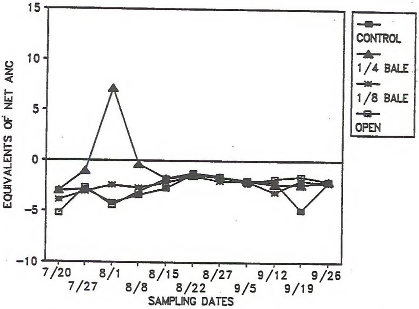


Figure 54: Standing Stock of Net Acid Neutralizing Capacity (ANC) in Experiment 6.

Table 11: Wilcoxon Signed Rank Test on Experiment #6. Z statistics are listed.
* .05<P<.1 **.01<P<.05 ***P<.01

Temperature

	Contr.	3/4	1/2	3/8	1/4	1/8	Open
Contr.		-1.5	-0.46	-0.46	-0.09	-1.3	-0.89
3/4			-1.7 *	-2.05 **	-0.8	-0.67	-0.68
1/2				-0.68	-0.66	-1.42	-0.99
3/8					-0.28	1.57	-1.17
1/4						-1.78 *	-1.56
1/8							-0.59
Open							

Sulfide

	Contr.	3/4	1/2	3/8	1/4	1/8	Open
Contr.		-2.6 ***	-4.01 ***	-0.35 ***	-2.35 **	-2.05 **	-1.05
3/4			-1.02	-0.16	-2.6 ***	-2.8 ***	-2.35 **
1/2				-2.29 **	-3.63 ***	-4.01 ***	-3.71 ***
3/8					-3.15 ***	-3.52 ***	-3.25 ***
1/4						-2.93 ***	-1.98 **
1/8							-0.94
Open							

Oxygen

	Contr.	3/4	1/2	3/8	1/4	1/8	Open
Contr.		-4.62 ***	-4.88 ***	-4.92 ***	-4.88 ***	-4.17 ***	-2.22 **
3/4			-0.07	-0.54	-3.4 ***	-3.6 ***	-4.7 ***
1/2				-1.36	-3.6 ***	-3.74 ***	-4.91 ***
3/8					-3.82 ***	-4.02 ***	-4.99 ***
1/4						-3.1 ***	-4.96 ***
1/8							-4.77 ***
Open							

Iron

	Contr.	3/4	1/2	3/8	1/4	1/8	Open
Contr.		-3.09 ***	-1.45	-1.82	-2.25 **	-2.39 **	-2.48 **
3/4			-2.31 **	-1.85 *	-2.17 **	-1.01	-1.26
1/2				-1.33	-0.34	-0.3	-0.22
3/8					-0.66	-0.36	-0.13
1/4						-0.03	-1.05
1/8							-0.94
Open							

pH

	Contr.	3/4	1/2	3/8	1/4	1/8	Open
Contr.		-4.22 ***	-4.44 ***	-4.22 ***	-3.81 ***	-3.18 ***	-4.62 ***
3/4			-2.31 **	-1.76 *	-2.43 **	-4.11 ***	-2.7 ***
1/2				-2.81 ***	-3.05 ***	-4.35 ***	-4.03 ***
3/8					-2.94 ***	-4.13 ***	-3.47 ***
1/4						-3.19 ***	-1.44
1/8							-0.73
Open							

Alkalinity

	Contr.	3/4	1/2	3/8	1/4	1/8	Open
Contr.		-2.52 **	-3.72 ***	-3.73 ***	-2.67 ***	-1.34	0
3/4			-2.48 ***	-1.48	-2.42 *	2.52 *	-2.52 *
1/2				-3.42 ***	-3.54 ***	-3.72 ***	-3.72 ***
3/8					-3.72 ***	-3.72 ***	-3.72 ***
1/4						-2.67 ***	-2.66 ***
1/8							-1.34
Open							

Sulfate

	Contr.	3/4	1/2	3/8	1/4	1/8	Open
Contr.		-0.52	-1.63	-1.45	-1.3	-0.65	-0.13
3/4			-0.87	-1.06	-2.07 **	-0.87	-0.62
1/2				-0.57	-0.26	-0.76	-1.42
3/8					-0.38	-0.57	-1.7 *
1/4						-1.23	-1.12
1/8							-0.86
Open							

Acidity

	Contr.	3/4	1/2	3/8	1/4	1/8	Open
Contr.		-1.48	-2.82 ***	-2.48 **	-2.56 **	-0.84	-2.01 **
3/4			2.32 **	-0.89	-0.87	-2.61 ***	-0.3
1/2				-2.06 **	-2.07 **	-3.62 ***	-2.52 **
3/8					-0.69	-3.01 ***	-1.72 **
1/4						-3.48 ***	-0.53
1/8							-1.38
Open							

Table 11: Continued

Zinc

	Contr.	3/4	1/2	3/8	1/4	1/8	Open
Contr.		-3.22 ***	-3.87 ***	-2.99 ***	-2.32 **	-2.49 **	-3.45 ***
3/4			-1.5	-0.12	-2.65 ***	-2.5 **	-2.2 **
1/2				-1.51 *	-3.57 ***	-3.2 ***	-3.23 ***
3/8					-1.92 *	-1.59	-2.33 **
1/4						-0.62	-1.02
1/8							-0.7
Open							

Manganese

	Contr.	3/4	1/2	3/8	1/4	1/8	Open
Contr.		-1.83 *	-1.31	-0.88	-1.26	-1.71 *	-0.94
3/4			-1.44	-0.19	-0.01	-1.43	-0.46
1/2				-0.01	-0.46	-1.79 *	-0.41
3/8					-0.75	-1.44	-0.79
1/4						-3.66 ***	-0.39
1/8							-2.31 **
Open							

Aluminum

	Contr.	3/4	1/2	3/8	1/4	1/8	Open
Contr.		-1.8 *	-3.45 ***	-3.89 ***	-4.03 ***	-4.17 ***	-2.86 ***
3/4			-1.15	-1.78 *	-1.12	-0.31	-0.36
1/2				-1.02	-1.1	-1.76 *	-1.67 *
3/8					-2.08 **	-2.8 ***	-2.87 ***
1/4						-1.55	-2.06 **
1/8							-0.92
Open							

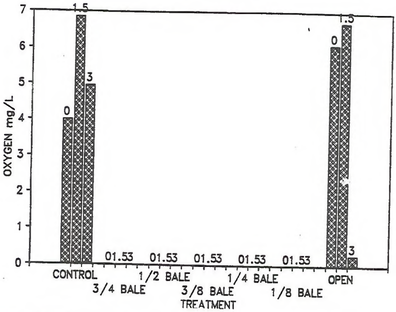


Figure 55: Comparisons of oxygen concentrations among treatments in Experiment 6 on August 1, 1989 Numbers on columns are depths in enclosures.

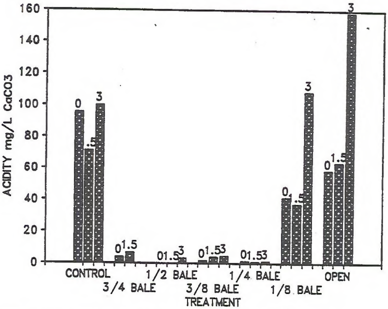


Figure 56: Comparisons of acidity among treatments in Experiment 6 on August 1, 1989. Numbers on columns represent depths in the enclosures.

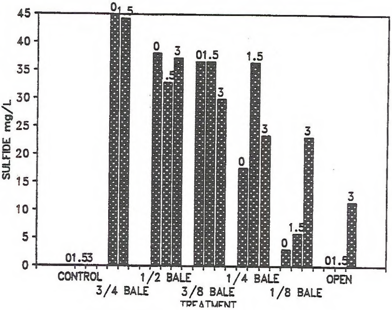


Figure 57: Comparisons of sulfide concentrations among treatments in Experiment 6 on 8/1/89. Numbers on columns are depths in enclosures.

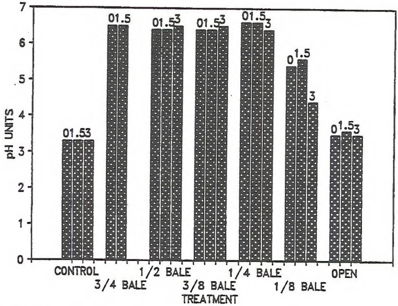


Figure 58: Comparisons of pH among treatments in Experiment 6 on 8/1/89. Numbers on columns represent depths in the experimental enclosure.

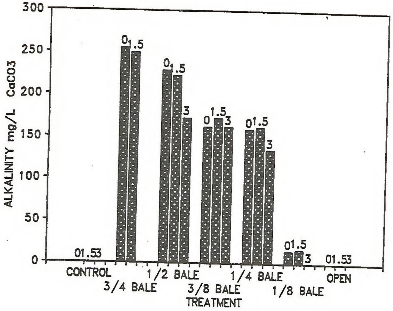


Figure 59: Comparisons of alkalinity among treatments during Experiment 6 on 8/1/89. Numbers on columns are depths in enclosures.

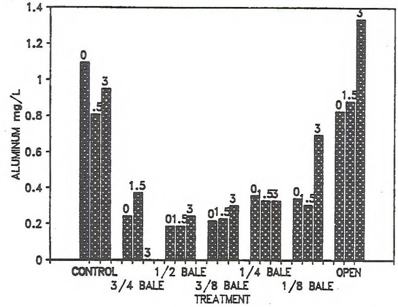


Figure 60: Comparisons of aluminum concentrations among treatments in Experiment 6 on 8/1/89. Numbers on columns represent sample depths in the experimental enclosures.

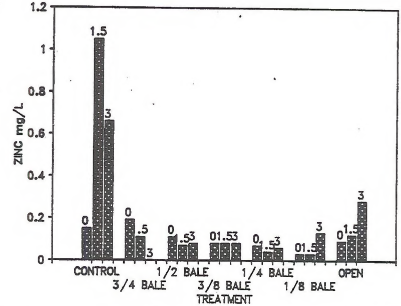


Figure 61: Comparisons of zinc concentrations among treatments in Experiment 6 on 8/1/89. Numbers on columns are depth in the experimental enclosures.

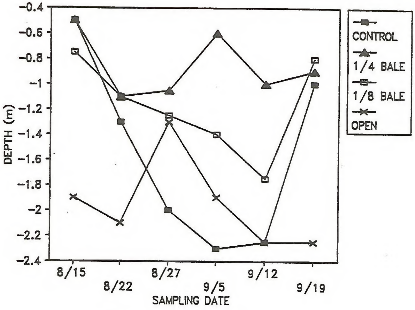


Figure 62 : Variations in Secchi disc transparency in enclosures during Experiment 6.

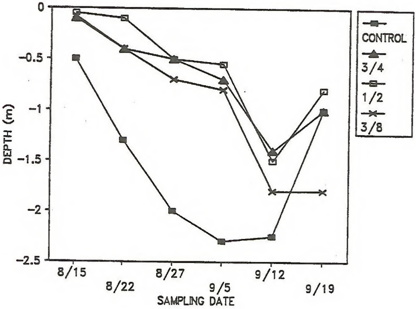


Figure 63 : Variations in Secchi disc transparency in enclosures during Experiment 6.

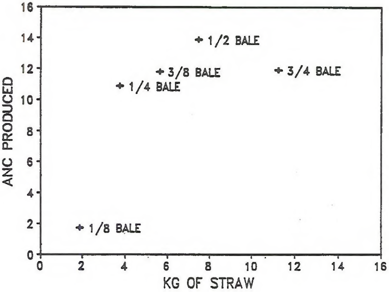


Figure 64: Comparisons between amounts of straw added to an enclousre and the maximum amount of Acid Neutralizing Capacity generated.

Secchi disc depths were shallow in the enclosures that received larger loadings of straw (Figs. 62 and 63). As with experiment 5, the added straw produced dark, turbid water. Enclosures with smaller amounts of straw were as clear as the control enclosure and the open lake. The turbidity of the enclosures declined during the course of the experiment.

Discussion - Experiment 6

In this experiment we tried to estimate the amount of straw needed to produce a certain amount of ANC. Such an estimate would be valuable to reclamationists in the application of our ideas.

The maximum ANC produced in an enclosure was graphed against the weight of straw added to the enclosure (Fig. 64). There appears to be a linear relationship between ANC and weight of straw added. The 3/4 bale enclosure represents an exception to linearity but at the end of the experiment we found that this enclosure had come lose from the sediment allowing acidic water to enter. Thus, only the 1/8 to 1/2 bale enclosures properly show the relationship between organic loading rates and ANC generation. The result of this analysis is that each kilogram of straw produces 2 equivalents of ANC, over a 10-week period.

Experiment 6 showed that neutralization is not permanent. The enclosures neutralized, but as soon as H_2S disappeared, they became acid again. It is not clear whether the re-acidification was a result of the leakage of lake water through the enclosure walls or whether the reinvasion of oxygen into the enclosures reoxidized dissolved H_2S in the water column to H_2SO_4. The former process would not be a problem in an open lake, but the latter certainly would be.

GENERAL CONCLUSIONS

The Role of Iron in the Neutralization Process

The results of our experiments contrast with those of some other investigators. In our experiments, neutralization did occur, but was transient. The pH rose and acidity declined in organically-treated enclosures as a result of sulfide production, but when the enclosures became aerobic again, pH declined and acidity rose. These results stand in contrast to those of other investigators.

Schindler *et al*. (30, 31) found that the sediment of acid precipitation-influenced lakes caused permanent neutralization by a combination of sulfate reduction and FeS precipitation. Likewise, Herlihy and Mills (15) and Herlihy *et al*. (16) found that sulfate reduction effectively removed acidity and metals from an acid mine drainage-influenced stream that entered a reservoir. Finally, Davison (8) treated an acid sand-pit lake with lime and organic matter. In his case, neutralization and removal of metal contamination lasted two years.

The major functional difference between the results of the other investigators and ours is the decline in dissolved iron seen by others. In the case of Herlihy and Mills (15) there were increases in "acid volatile sulfide" (probably mostly FeS) in the lake sediment. Our results show no declines in iron when organic matter is added to pit #8. The only clear declines came where lime alone was added to the enclosures.

If Anderson and Schiff (2) are correct that permanent neutralization occurs only when both sulfate and Fe^{+++} are reduced and precipitated as FeS, then permanent neutralization of our enclosures is unlikely because FeS precipitation did not occur. The temporary neutralization that occurred in our enclosures seems to have happened because of the presence of H_2S -- a good buffer. When reoxygenation of the water columns occurred, the H_2S was reoxidized, releasing mineral acidity.

A reasonable question is, "How are Pit #8 and our treatments different from the other places where sulfate reduction permanently neutralized lakes?" Three possible hypotheses can be constructed to answer this question.

The first hypothesis is that Pit #8 is more acid than the sites the other investigators used and that this prevents sulfate reduction and FeS precipitation. The Middle Incline of Pit #8 is essentially a pool of acid mine drainage whereas the areas studied by Schindler *et al*. (30) and Herlihy and Mills (15) were only slightly contaminated by acid when compared with Pit #8. Davison's (8) location was more like Pit #8, but he also added lime with the organic matter.

The higher acidity of Pit #8 is, however, unlikely to be a factor in preventing FeS precipitation because the pH's of the straw enclosures rose to levels where significant amounts of S^{--} would be available for FeS precipitation.

A second hypothesis to explain the transience of neutralization in our enclosures is that the enclosures leaked. It is likely that there was some interchange of water between the enclosures and the open lake. When the ANC curves for lime additions are examined (Figs 3, 6, 13, 30), it is apparent that lime additions quickly neutralized the enclosures, but that ANC declined over time. If there were no water interchange, the neutralization would have been permanent. A small amount of water must have entered the enclosures to replace water taken in sampling. A total of 60 liters was taken from each enclosure over the 10 week experiment and open lake water entered to replace it. Enclosure volumes varied from 3,100 to 4,700 liters. We believe that a small amount of water also entered the enclosures through the seal between the polyethylene and the lake sediment.

We do not believe that leaks in the enclosures were the primary cause for the rapid acidification of the enclosures, however. If leaks had been significant, sulfate reduction could not have occurred because the constant influx of oxygen-rich water. It is evident that the enclosures were sealed enough to permit sulfate-reduction to occur. In addition, the presence of leaks does not explain the increases in iron which occurred in the lime/straw and straw enclosures during experiment 5 (Figs. 49 and 50). Levels of iron in these enclosures far surpassed the open lake. We think that the failure to remove iron from the water column as an FeS precipitate was the cause of the reacidification of the enclosures rather than an influx of acidic open lake water through leaks in the enclosures.

A third and most plausible hypothesis to explain the reacidification of our enclosures is that the large amounts of straw resulted in high concentrations of dissolved organic matter in the water columns. In natural lakes, decomposing organic matter produces humic acids which chelate iron (18). If such chelators exist in our enclosures, then they would keep iron in solution even though the solubility product of FeS seems to be exceeded. As a result, sulfide precipitation and permanent neutralization are blocked.

Evidence for the third hypothesis comes from Experiment 5. In this experiment, iron concentrations actually increased with time in the straw enclosure (Fig. 50) suggesting that FeOOH reduction and dissolution was occurring as described by Anderson and Schiff (2). Lime treatment removed iron from the water column. Lime/straw treatments were less effective in iron removal than lime alone, suggesting that the postulated chelating agents released by the straw reduce iron precipitation by lime as well as by sulfide.

The Fate of Reduced Iron and Sulfur in an Organically Loaded Lake

Our experiments clearly show that sulfate reduction alone is not sufficient to ensure the permanent neutralization of a surface mine lakes. The Fe^{++} and S^{--} produced by dissimilatory reduction must be removed from the water column. If they are not removed, they remain in the water column as mineral acidity and will be reoxidized later to produce large amounts of H^{+}. In lakes where sulfate reduction has been shown to cause permanent neutralization (30,31,15,16), the precipitation of FeS was strong. In our enclosures neither Fe^{++} nor S^{--} was removed from the water in any significant amount. In some enclosures like the straw enclosure of experiment 5 (Fig. 50), iron was actually mobilized by the low redox potential. The solubility product of FeS was far exceeded in that enclosure.

Our results show that in any system for removing sulfuric acid by sulfate reduction care must be taken to determine the fate of the Fe^{++} and S^{--} that are produced. They must be removed from the water column if permanent neutralization is to occur.

The Role of Organic Matter in the Natural Recovery of Mine Lakes

Our experiments represent a test of King *et al.*'s (22) suggestion that acidic surface mine lakes neutralize over time because the accumulation of decomposable organic matter from a revegetating watershed supports increased dissimilatory sulfate reduction in the deeper layers of the water column. Our estimate that one kilogram of straw produces 2 gram equivalents of ANC allows us to calculate the amount of added organic matter that is needed for the neutralization of a lake.

If a hypothetical lake is 100 m^2 and 3 m deep with an acidity of 50 mg/l $CaCO_3$ (1 meq/l) then 7500 kg of straw would be needed for neutralization. Although this is a large amount of organic matter, it could be supplied by the organic litter from a watershed of reasonable size. Jordan (21) presents data on the production of organic litter in various ecosystems. For temperate zone terrestrial environments, litter production is about 250 $gm/m^2/yr$. If this production rate applies to the watershed surrounding our hypothetical lake, then to neutralize the lake, a watershed area of 30 ha. (74 acres) would be required.

Thus, it is possible that the litter production from a revegetated surface-mined landscape would be adequate to neutralize a small lake. Problems will occur in unrevegetated landscapes where litter production is low and in large, very acid lakes where large amounts of ANC are required for neutralization. Neutralization by naturally-supplied organic matter is most likely in small, weakly acidic lakes. It is likely that the natural neutralization process occurs as a result of a combination of factors which include the accumulation of decomposing organic matter in the lake. Another factor in lake neutralization will be the decline in acid production from a watershed where pyrite is being weathered away.

The Use of Artificial Organic Matter Additions to Encourage Neutralization

Our experiments show that the H_2S itself is the source of the neutralization. When it disappears, pH goes down. This result is not unexpected because H_2S is a strong buffer.

A problem with the wheat straw we added is that it is inefficient in removing metals from the water column. Zinc and aluminum were effectively removed, but iron and manganese were not. This result is a paradox because we would expect iron to precipitate as a sulfide. It may be, as suggested above, organic substances from the straw act as chelators to hold metals in solution.

Another problem with neutralization by organic addition is its short duration. Because our experiments show only transitory neutralization, it is likely that efficient Fe^{+++} reduction and precipitation is not occurring. We see no decline in water column Fe so there is probably little FeS precipitation.

An alternative to FeS precipitation for permanent removal of H^+ from the water column is outgassing of H_2S (8). Apparently, this process was not vigorous enough in our enclosures to remove significant amounts of acidity. It was the major process neutralizing King *et al.*'s (22) microcosms. Outgassing of H_2S that is vigorous enough to remove significant acidity from the water column might cause other environmental problems because H_2S is a foul-smelling poisonous gas that discolors painted objects.

Another problem with the neutralization of an acid mine lake with organic matter is the poor quality of water that results. The low oxygen and high sulfide levels are very toxic to most multicellular organisms. The resulting water is turbid and smells of sulfide. It is probably inappropriate to treat a whole lake with organic matter in a way similar to ours because of the low water quality that results. To solve this problem, the organic matter might be confined to deep water where the degradation of water quality might not be so obvious.

A final problem with the technique is the large amount of straw required. In our experiments only 2 gram equivalents of ANC were produced for each kilogram of straw added to the enclosures. In contrast, $CaCO_3$ produces 20 gram equivalents of ANC per kilogram. If all the organic matter in the straw were completely oxidized according to equation c, then 33 gram equivalents of ANC would be produced per kilogram of straw. It is evident that the full potential of the straw for supporting dissimilatory sulfate reduction was not seen in our experiments. We noted large quantities of undecomposed straw remaining at the end of each experiment so it is likely that oxidation of the straw was incomplete. Straw may not be the material of choice to support sulfate reduction. A more labile organic substance might be used instead.

The large amounts of straw required for neutralization might not be a problem for a mine operator if the cost of the straw was less than the cost for alternative chemical neutralizing materials. Other organic materials like agricultural or sewage wastes might cost less.

RECOMMENDATIONS

Although there are problems with the addition of straw directly to lakes for the purpose of neutralization, these problems can probably be solved by using a different organic material and different application methods. The broader potential of organic matter as a medium for neutralization is strong.

The first modification in the method is to use a different source of organic matter. Although straw is an analogue for natural plant material, it does not decompose quickly enough to produce the large amounts of ANC needed for neutralization. A more decomposable material would be more efficient. The organic matter should also produce few organic chelators to inhibit the precipitation of FeS.

Another problem with straw is its bouyancy. It tends to begin decomposition in the water column, causing water quality problems. An alternative source of organic matter would have to sink. Anaerobic decomposition is more likely to occur at the sediment/water interface. Here is where dissimilatory sulfate reduction will be most vigorous and the organic matter will be most effectively decomposed. This location will also minimize the degradation of open lake water quality.

Many midwestern surface mine lakes are highly stratified with very anaerobic deep layers (35). In particular lakes, these layers may be the result of thermal stratification as in a natural lake or they may result from chemical stratification as in Pit #8. These stratified lakes should be the best candidates of all for organic matter neutralization because the organic material could be piped as a slurry directly into the deep, anaerobic layers where they will support high rates of dissimilatory sulfate reduction. The long-term stratification should allow the reactions that Anderson and Schiff (2) describe. The long period of stratification should allow time for FeS deposition. Our enclosures were insufficiently shielded from reoxidation. Because reoxidation is less likely in the deep water of a stratified lake, the effects of sulfate reduction are more likely to be permanent.

Adding the organic matter to the deep water of the lake will reduce water quality problems at the lake surface. The deep water should build up ANC throughout the period of summer stratification and this high pH water should be distributed through the water column in fall as the lake destratifies.

Our experiments and those of Davison (8) suggest that lime additions will encourage neutralization by organic matter. For neutralization to occur it may be necessary to add small amounts of lime to the stratified layer in addition to the organic material.

In conclusion, organic additions to surface mine lakes show potential for neutralization, but modifications in application procedures must be tested before the techniques can be routinely used.

REFERENCES

1. American Public Health Association. Standard Methods for the Examination of Water and Wastewater., 1985, 874pp.
2. Anderson, R.L. and S.L. Schiff. 1987. Alkalinity generation and the fate of sulfur in lakes sediments. Can. J. Fish. Aquat. Sci., v. 44(Suppl. 1), 1987, pp. 188-193
3. Buchanan, R.E. and N.E. Gibbons. Bergey's Manual of Determinative Bacteriology. The Williams and Wilkins Co. Baltimore, 1974, 1268 pp.
4. Baas-Becking, L.G.M., I.R. Kaplan, and D. Moore. Limits of the natural environment in terms of pH and oxidation-reduction potentials. Journal of Geology, v. 68, 1960, pp. 243-284.
5. Brugam, R.B., M.A. Carlson, S. Chakraverty, and M. Lusk. Post-mining neutralization of acidic surface mine lakes. Research Report #178. Water Resources Center University of Illinois, Urbana-Champaign, 1981, 96 pp.
6. Campbell, R. S. and O.T. Lind. Water quatlity and aging of strip-mine lakes. J. Wat. Pollut. Cont. Fed., v. 41, 1969, pp. 1943-1955.
7. Corbett, C. Acid mine drainage problems of the Patoka River watershed, southwestern Indiana. Indiana University Water Resources Center. Report of Investigations No. 4., 1969, pp. 1-173.
8. Davison, W. Sewage sludge as an acidity filter for groundwater-fed lakes. Nature, v, 322, 1986, pp 820-822.
9. Difco. Difco Manual. Tenth Edition. Difco Laboratories. Detroit, Michigan, 1984, 1155 pp.
10. Gibb, J.P. and R.L. Evans. Preliminary evaluation of final cut lakes. Illinois State Water Survey. Urbana. Circular No. 130, 1978, pp 1-87.
11. Guillard, R.R.L. Culture of phytoplankton for feeding marine invertebrates. In:Culture of Marine Invertebrates. W.L. Smith and M.H. Chanley eds. Plenum, New York., 1975, pp. 29-60.
12. Gyure, R.A., A. Knopka, A. Brooks, and N. Doemel. Algal and bacterial activities in acidic (pH 3) strip mine lakes. Applied and Environmental Microbiology, v. 53, 1987, pp 2069-2076.

13. Havens, K. E. and J. DeCosta. The effects of acidification and phosphorus addition on phytoplankton biomass and productivity in a circum-neutral mesotrophic lake receiving acid precipitation. Arch. Hydrobiol. Suppl Bd., v. 69, 1984, pp. 443-476.

14. Haynes, R.J. and W.D. Klimstra. Illinois Lands Surface Mined for Coal. Cooperative Wildlife Research Laboratory, Southern Illinois University, Carbondale, 1978, 201 pp.

15. Herlihy, A.T. and A.L. Mills. Sulfate reduction in freshwater sediments receiving acid mine drainage. Applied and Environmental Microbiology, v. 49, 1985, pp. 179-186.

16. Herlihy, A.T., A.L. Mills, G.M. Hornberger and A.E. Bruckner. The importance of sediment sulfate reduction to the sulfate budget of an impoundment receiving acid mine drainage. Water Resources Research, v. 23, 1987, pp. 287-292.

17. Hood, W.C., T.A. Delfino, and P.D. Robinson. Acid leachable metals in streams. In: MiniSymposium, Elements in Coal and Potential Environmental Concerns Arising from those Elements. Society of Mining Engineers of AIME. Coal Series 79-06, 1979, pp 15-20.

18. Hutchinson, G.E. Treatise on Limnology Vol. I. John Wiley and Sons, Inc. New York, N.Y., 1957, 1015 pp.

19. Hutchinson, G.E. Eutrophication: The scientific background of a contemporary practical problem. Amer. Sci., v. 61, 1973, pp. 269-279.

20. Jones, D.W., M.J. McElligott, and R.H. Mannz. Biological, Chemical and Morphological Characterization of 33 Surface mine Lakes in Illinois and Missouri. Peabody Coal Co., Fairview Heights, Ill., 1985, 248 pp.

21. Jordan, C. F. A world pattern in plant energetics. American Scientist, v. 59, 1971, pp. 425-433.

22. King, D. L., J.J. Simmler, C.S. Decker and C.W. Ogg. Acid strip mine lake recovery. Journal of the Water Pollution Control Federation, v. 46, 1974. pp. 2301-2316.

23. Klimstra, W.D. and J. R. Nawrot. Water as a reclamation alternative: An assessment of values. In: Symposium on Surface Mining, Hydrology and Reclamation. Donald Graves, ed. University of Kentucky., 1982, pp. 39-44.

24. Konik, J. Some physical, chemical and biological characteristics of non-problem waters occuring on lands surface mined for coal. Document 80/14 Illinois Institute of Natural Resources, Environmental Management Division, Chicago, IL., 1980. 90 pp.

25. Lund, J.W.G. Investigations on phytoplankton with special reference to water usage. Freshwater Biological Association of the U.K. Occasional Paper No. 13, 1981. pp 3-64.

26. McKnight, D. B.A. Kimball, K.E. Bencala. Iron photoreduction and acidations in an acidic mountain stream. Science, v. 240, 1988, pp. 637-640.

27. Pfennig, N. & F. Widdel. The bacteria of the sulfur cycle. Phil. Trans. Roy Soc. B., v. 298, 1982, pp. 433-441.

28. Porter, K.G. 1976. Enhancement of algal growth and productivity by grazing zooplankton. Science, v. 192, 1976, pp. 1332-1334.

29. Schiff, S.L. and R.F. Anderson. Limnocorral studies of chemical and biological acid neutralization in two freshwater lakes. Can. J. Fish. Aquat. Sci., v. 44(Suppl), 1987. pp 173-187.

30. Schindler, D.W. and R. Wagemann, R.B. Cook, T. Ruszcynski, and J. Prokopowich. 1980. Experimental acidification of Lake 223, Experimental Lakes Area: Background data and the first three years of acidification. Can. J. Fish. and Aquat. Sci., v. 37, 1980, pp 342-354.

31. Schindler, D.W., M.A. Turner, M.P. Stainton, and G.A. Linsey. Natural sources of acid neutralizing capacity in low alkalinity lakes of the Precambrian Shield. Science , v. 232, 1986, pp. 844-847.

32. Schuurkes, J.A.A.R. and C.J. Kok. In vitro studies on sulfate reduction and acidification in sediments of shallow soft water lakes. Freshwater Biology, v. 19, 1988. pp 417-426.

33. Schwoerbel, J. Methods of Hydrobiology. Pergamon Press, New York, 1970, 200 pp.

34. Shapiro, J. Blue green algae: Why they become dominant?. Science, v. 179, 1973, pp. 382-384.

35. Smith, R.W. and D.G. Frey. Acid-Mine Pollution Effects on Lake Biology U.S.E.P.A. Project #18050EED.Washington, D.C., 1971, 131 pp.

36. Stahl, J.B. 1978. Water and peculiar stratification in an organically loaded strip-mine lakes. Water Research, v. 13, 1978. pp 467-471.

37. Stumm, W. and J.J. Morgan. Aquatic Chemistry: An Introduction Emphasizing Chemical Equilibria in Natural Waters. Wiley-Interscience, New York., 1970, 583 pp.

38. Sokal, R. B. and F.J. Rohlf. Biometry: Principles and Practise of Statistics in Biological Research W.H. Freeman & Co. San Francisco, 2d ed., 1981, 875 pp.

39. Wetzel, R. Limnology Saunders College Publishing, Philadelphia, 2d ed., 1983, 763 pp.

40. Wilcox, G. and J. DeCosta. The effect of phosphorus and nitrogen addition on the algal biomass and species composition of an acidic lake. Arch. Hydrobiol. v. 94, 1982, pp. 393-424.

Reproduced by NTIS
National Technical Information Service
U.S. Department of Commerce
Springfield, VA 22161

This report was printed specifically for your order from our collection of more than 2 million technical reports.

For economy and efficiency, NTIS does not maintain stock of its vast collection of technical reports. Rather, most documents are printed for each order. Your copy is the best possible reproduction available from our master archive. If you have any questions concerning this document or any order you placed with NTIS, please call our Customer Services Department at (703)487-4660.

Always think of NTIS when you want:

- Access to the technical, scientific, and engineering results generated by the ongoing multibillion dollar R&D program of the U.S. Government.
- R&D results from Japan, West Germany, Great Britain, and some 20 other countries, most of it reported in English.

NTIS also operates two centers that can provide you with valuable information:

- The Federal Computer Products Center - offers software and datafiles produced by Federal agencies.
- The Center for the Utilization of Federal Technology - gives you access to the best of Federal technologies and laboratory resources.

For more information about NTIS, send for our FREE *NTIS Products and Services Catalog* which describes how you can access this U.S. and foreign Government technology. Call (703)487-4650 or send this sheet to NTIS, U.S. Department of Commerce, Springfield, VA 22161. Ask for catalog, PR-827.

Name ______________________________
Address ______________________________
____
____
Telephone ______________________________

- Your Source to U.S. and Foreign Government Research and Technology.

NTIS does not permit return of items for credit or refund. A replacement will be provided if an error is made in filling your order, if the item was received in damaged condition, or if the item is defective.

88044015

R'S CARD

990a

acid mine
ced lakes

OFFICE

DATE RETURNED

(Continued on reverse)

TD 899 .M5 B783 1990a

Neutralization of acid mine
drainage influenced lakes

U.S. DEPARTMENT OF COMMERCE
Technology Administration
National Technical Information Service
Springfield, VA 22161   (703) 487-4650